



MADROÑO

A WEST AMERICAN JOURNAL OF BOTANY

DEDICATION

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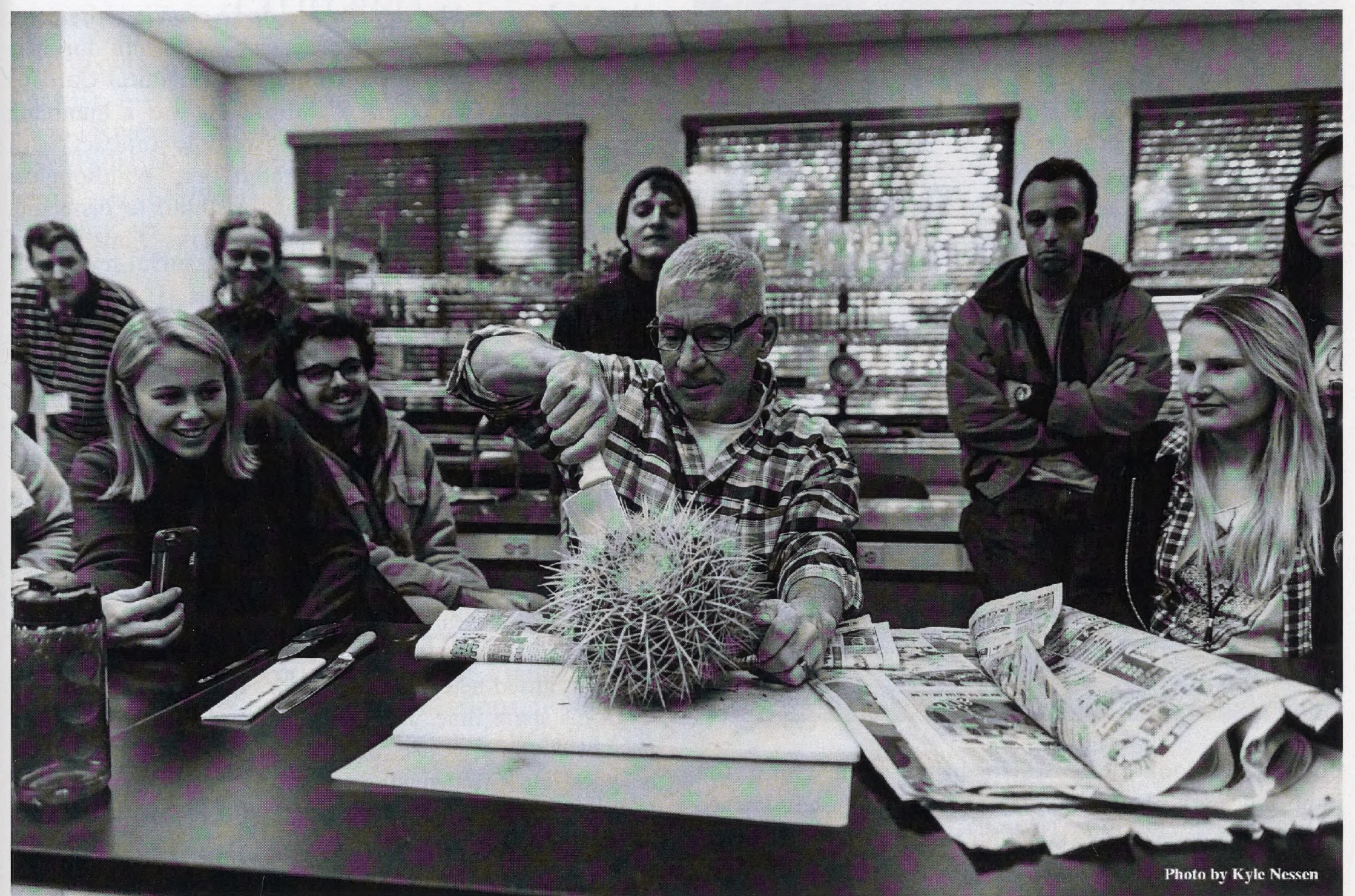


Photo by Kyle Nessen

In 2017, the California Botanical Society is pleased to dedicate the 64th volume of *Madroño* to Dr. James (Jim) D. Mauseth, author, renowned plant anatomist, celebrated teacher, and botany professor at the University of Texas at Austin (UT).

In the introduction to the sixth edition of *Botany: An Introduction to Plant Biology*, Jim writes: “At this point in human history, we must look at plants in a new way, we must analyze the interactions of plants and people more carefully than ever before.” For forty-one years, Jim has devoted his life to training students to do just that.

Jim Mauseth was born in 1948 in eastern Washington. He grew up surrounded by plants on his family farm and the nearby sagebrush scrub. He received a bachelor’s degree and Ph.D. from the University of Washington, Seattle where he studied the growth and development of cacti. However, he didn’t initially go to grad school to study cacti. It took a serendipitous field trip to Anza Borrego for Jim to meet and fall in love with his first cactus. Since then he’s tackled the most fundamental questions about how plants adapt to extreme environments.

Jim became a professor at UT in 1975, and after forty-one years of incredible service, Jim retired this year. Much of his career has concentrated on studying plants with highly modified forms, such as cacti and parasitic plants. To

accomplish his research, Jim traveled extensively throughout Central and South America to study cactus diversification. He chronicled these adventures in a delightful book: *A Cactus Odyssey: Journeys in the Wilds of Bolivia, Peru, and Argentina* (Timber Press, 2002) where he brought the tribulations and excitement of botanical discovery to the masses.

Jim has made a career out of looking closely at plants. His anatomical studies have elucidated many new and poorly understood complexities about how plants work. His prolific publications include over seventy-five papers related to plant form. He also authored nearly thirty articles about plants and cacti for domestic and international societies. We are all indebted to the careful observations that Jim has contributed to our understanding of plant anatomy.

During his time at UT, Jim authored several renowned and widely used textbooks. His *Botany: An Introduction to Plant Biology* is now in its 6th edition (Jones & Bartlett Learning, 2016), and *Plant Anatomy* (Blackburn Press, 2008) still continues to serve upper division undergraduate courses throughout North America. All of Jim’s time traveling internationally and teaching at UT have given him a unique understanding of how dependent human life is on plants. To that end, Jim wrote *Plants and People* (Jones & Bartlett Learning, 2012), an ethnobotany and economic

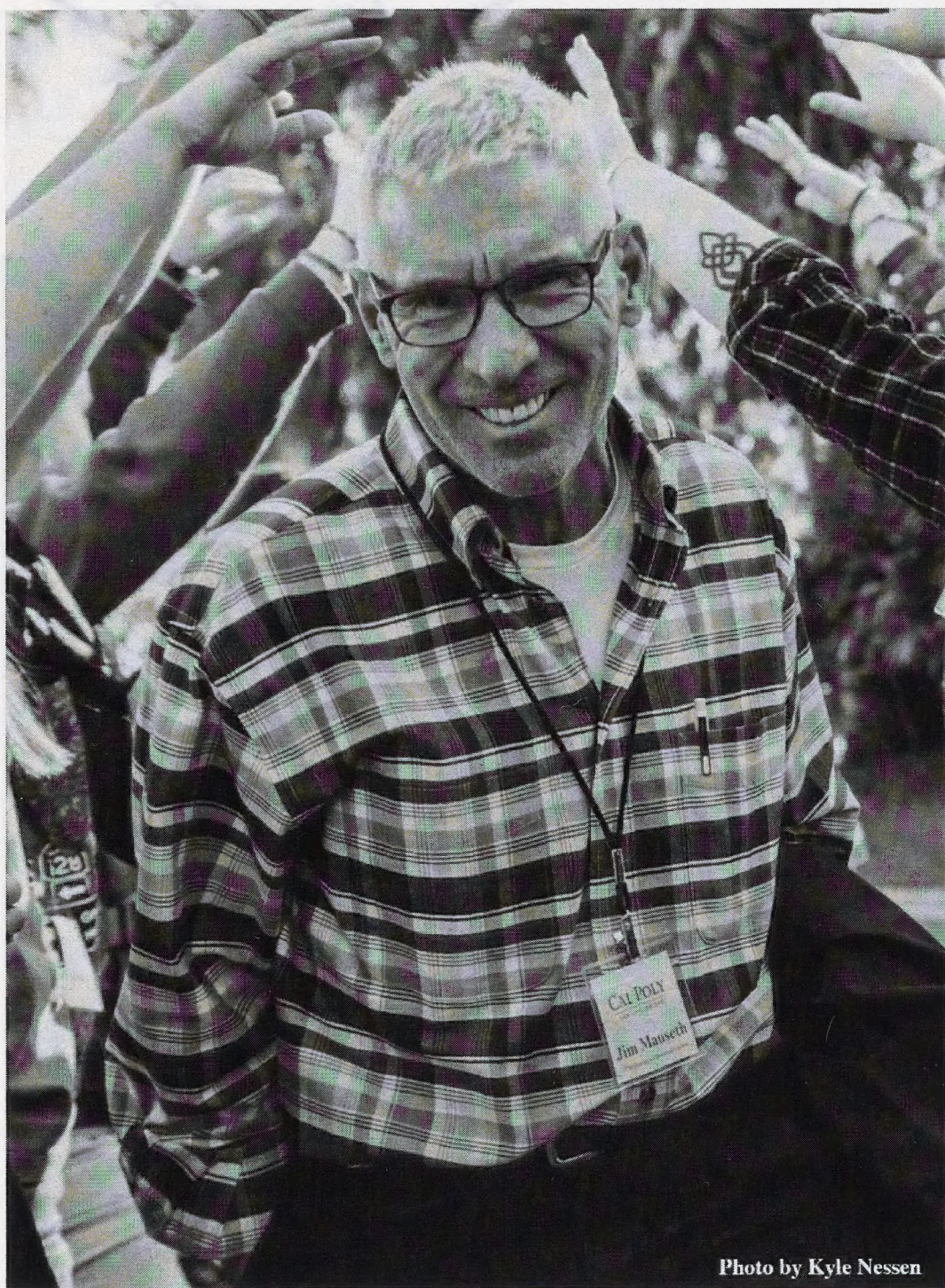


Photo by Kyle Nessen

botany textbook designed to bring science and the importance of plants in human cultures to non-science majors. Jim has a unique talent for making textbook science writing interesting to wide audiences.

Jim has impacted countless students in his own courses at UT. He's taught Introductory Botany and Plant Anatomy nearly every year since 1975, as well as Plant Biotechnology, Plant Morphogenesis, and Structure and Function of Organisms. Jim has also taught extensively throughout Latin America with courses in Spanish including Plant Anatomy and Cacti Ecology and Evolution. Jim has also done his part in training the next generation of plant anatomists. His many graduate students have embarked on studies of *Mammillaria* Haw., *Physcomitrium* (Brid.) Brid., the Balanophoraceae, the Caryophyllales, and of course cacti in general.

Jim is really at his best in front of a group of students, and the students hang on every word. At any moment Jim can deliver an impromptu lecture on a wide range of topics. Jim is funny, sharp, and deeply caring. He's inspired hundreds, if not thousands, of students to take an interest in plant biology, and he continues to do so even in retirement. We recently had the honor of teaching with him during a short course on cacti at the Huntington Botanical Gardens and Library, in Pasadena, California. It was a humbling experience to watch him so masterfully interact with students, from teaching them about deep philosophical issues associated with the scientific method, to dissecting cacti, to explaining how a microtome works. Jim's love of plants and scientific discovery is infectious.

Jim currently serves as the editor of the Cactus and Succulent Society of America's journal, *Haseltonia*, which features peer-reviewed articles about all aspects of cacti, succulents, and their environs. He's been a contributing member of the Cactus and Succulent Societies of America and Great Britain, the International Association for Wood Anatomists, and the Botanical Society of America, just to name a few. He's served on the board of directors of the Texas Botanical Garden Society since 1995, been the associate editor of the *American Journal of Botany*, and on the editorial board of the *Plant Science Bulletin*. Jim has exemplified all that one could hope for as a productive scientist, gifted teacher, and supportive colleague.

In his spare time, Jim is an avid cyclist and enjoys the pace of life on a bicycle, where he can look at the plants as he goes. He's ridden all over the U.S. and Europe, and hopefully many more bike trips are in his future retirement plans.

Jim ends the preface of his *Botany* textbook with "My ultimate goal is to teach about life in general...to help the reader to more fully understand human biology, indeed to understand all of biology. No organism exists isolated from others: instead we all share one biology that encompasses all organisms. We are all in this together." Thank you Jim, from your many students and colleagues, for your important lifelong work, inspirational teaching, and for continuing to remind us to look closely at the botanical wonders surrounding us.

—JENN YOST AND MATT RITTER, Biological Sciences Department, California Polytechnic State University, San Luis Obispo, CA 93407

NOTEWORTHY COLLECTION

CALIFORNIA

TORTELLA HUMILIS (Hedw.) Jenn. (POTTIA-CEAE). — Ventura County, San Nicolas Island, Twin Rivers Canyon, elev. 10 m, 33.23207° N, 119.51808° W, 7 February 2016, B. E. Carter 9187, with C. M. Williams & W. Hoyer (CAS, MO, SJSU). Growing on north facing bank of dry wash covered in lichen crusts and thalloid liverworts (*Asterella californica* [Hampe ex Austin] Underw., *Targionia hypophylla* L.), in open, arid scrub dominated by *Leptosyne gigantea* Kellogg with *Dichelostemma capitatum* (Benth.) Alph. Wood, *Eriogonum grande* Greene, *Isocoma menziesii* (Hook. & Arn.) G.L. Nesom, and *Lomatium insulare* (Eastw.) Munz.

Previous knowledge. *Tortella humilis* is a widespread moss species, occurring in a wide range of habitats on all continents except Australia and Antarctica. In North America, the species is not uncommon throughout the eastern deciduous forests and the southeast. West of the Great Plains, it was previously known only from collections in British Columbia, Washington, and Arizona (Eckel 1998, 2007).

Significance. This collection, confirmed by P. Eckel, is the first record of *Tortella humilis* in California. The plants were without sporophytes but were healthy and locally common in an undisturbed area of approximately 50 m². The associated lichens and vascular plant species, including *Lomatium insulare* and *Dichelostemma capitatum*, are not common in Twin Rivers Canyon, which is on the arid south side of San Nicolas Island. Similar associations are more typical of the relatively more mesic northern and eastern regions on the island.

These areas were surveyed, but no additional populations of *T. humilis* were discovered.

Bryophyte endemism on the Channel Islands is very low, but the islands harbor many species that are uncommon or absent on the adjacent mainland (Carter 2015). These include a number of species that appear to be relicts, like those associated with the Bishop Pine forests of the northern islands, as well as more arid species (e.g., contributors to soil crusts) that were likely more common on the mainland prior to the human development of the Los Angeles region. While many of the isolated island populations can be explained by relictualism, bryophytes are also very good dispersers, and it is impossible to rule out long distance dispersal in accounting for these distributions. With only the single known population of *T. humilis* in southern California, the biogeographic history of the species in the region remains unknown.

—BENJAMIN E. CARTER, Department of Biological Sciences, San Jose State University, San Jose, California 95192; benjamin.carter@sjsu.edu.

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NOTEWORTHY COLLECTION

OREGON

GALEOPSIS TETRAHIT L. (LAMIACEAE). — Columbia County, Clatskanie, near the intersection of Hermo Road and Collins Road, established in the understory of a hybrid poplar plantation, 46.157433° N 123.179860° W, 2 August 2016, *Charles Bubl s.n.* (det. Abair) (OSC).

Previous knowledge. Common hemp-nettle is a coarse, taprooted annual, native to Eurasia. It can be found in waste sites and roadsides of Canada and adjacent states in the U.S., and the range extends through the Midwest and the Northeast down to North Carolina (Britton and Brown 1913) and Tennessee (Chester et al. 1997). Common hemp-nettle has also been collected in California (Hrusa et al. 2002), Idaho (CPNWH 2016), and Washington (CPNWH 2016). A specimen originally determined as *Galeopsis tetrahit* (Goodrich 148, ORE) collected in Linn County, Oregon was annotated to *Stachys rigida* by K. L. Chambers (OSC) in 1997 and J. B. Nelson (USCH) in 2016. The original determination is currently incorrectly mapped by the USDA Plant List (2016), the University of Montana Invaders Database (2016), and the Biota of North America Program (2016).

Significance. First collection for Oregon.

LYTHRUM TRIBRACTEATUM Salzm. ex Spreng. (LYTHRACEAE). — Jackson County, southwest bank of Emigrant Lake, along a creek that cuts deeply into the dried banks of the lake, elev 688 m, 42°08'22" N 122°37'14" W, 5 September 2016, *Abair 160905003* (OSC); off State Highway 66, about 4 air miles southeast of Ashland, at Emigrant Lake, below Cemetery Hill, elev 675 m, 42.14372° N 122.61968° W, associated taxa included *Bidens* L., *Cuscuta* L., *Euphorbia* L., *Mollugo* L., *Rorippa* Scop., and *Xanthium* L., 28 September 2016, *Halse 9870* (OSC); Off State Highway 66, about 4 air miles southeast of Ashland, at Songer Wayside, on southern end of Emigrant Lake, elev 671 m, 42.14178° N 122.59346° W, associated taxa included *Convolvulus* L., *Euphorbia*, *Mentha pulegium* L., *Polygonum* L., *Rorippa*, and *Xanthium*, 28 September 2016, *Halse 9874* (OSC).

Previous knowledge. Threebract loosestrife is a small, prostrate annual that has been introduced to North America from Europe. It is well-established in

California (Hickman 1993), and has been collected in Idaho (specimen at Albertson College of Idaho, Caldwell, ID), Nevada (Tiehm 2007), and Utah (Welsh et al. 2003).

Significance. First collection for Oregon. There are at least three established populations in the draw down zones of this reservoir (Emigrant Lake). Similar habitats across Oregon should be monitored for new populations of *Lythrum tribracteatum*.

—ALEXANDER L. ABAIR AND RICHARD R. HALSE, OSU Herbarium, Department of Botany and Plant Pathology, 2082 Cordley Hall, Corvallis, OR 97331-2902; abaira@oregonstate.edu.

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NOTEWORTHY COLLECTION

OREGON

CHAEROPHYLLUM TEMULUM L. (APIACEAE). — Washington County, Tualatin, dry soil, pasture along fence line (no geocoordinates provided), 29 June 2000, *White s.n.* (WTU, OSC).

Previous knowledge. Rough chervil is a large, biennial, taprooted European introduction of disturbed forest understory and margin, documented in eastern North America from Pennsylvania, New Jersey, and Quebec (Kartesz 2015). In western North America it is known from southwestern British Columbia and lowland western Washington (CPNWH 2015).

Significance. First collection for Oregon. Originally misidentified by the collector as *Anthriscus sylvestris* (L.) Hoffm. The documented range of *C. temulum* in the Puget Sound region of Washington has increased by more than 100 km in the last five years, suggesting that this species is more widespread than specimen records indicate. *Chaerophyllum temulum* is likely more widespread in the Willamette Valley of Oregon given that region's climatic and habitat affinities with the Puget Sound region.

CYPERUS FUSCUS L. (CYPERACEAE). — Columbia County, Columbia River, Trojan Park, just south of Coffin Rock, 4.5 air miles southeast of Rainier, elev. 3 m [inferred], 46.033849° N, 122.883311° W, 16 November 2013, *Otting 3723* (WTU).

Previous knowledge. Brown galingale is native to Eurasia, with introduced populations occurring in scattered areas of North America, including Ontario, Quebec, the New England states, South Dakota, Nebraska, Missouri, Nevada, and California (Tucker et al. 2002).

Significance. First collection for Oregon. The specimen was originally misidentified as *Cyperus difformis* L. Peter F. Zika, Cyperaceae expert, confirmed the specimen identification.

GALIUM PALUSTRE L. (RUBIACEAE). — Douglas County, Highway 99 between Interstate Highway 5 (I-5) and Drain, between the highway and a smaller road paralleling it to the north, elev. 110 m, 43.702827° N, 123.25116° W, 15 June 2013, *Wilson 17571* (WTU).

Previous knowledge. Common marsh bedstraw is native to eastern North America and Europe, and apparently introduced to southern British Columbia, Washington, and Montana (CPNWH 2015). It has been previously reported from Multnomah County, Oregon (University of Montana Invaders Database, <http://invader.dbs.umt.edu/>), although without a substantiating voucher or means of verification.

Significance. First verified collection for Oregon. The label indicated plants were “scrambling over other plants including *Carex scoparia* at edge of wetland.” The specimen was previously misidentified as *Galium trifidum* L.; *Galium palustre* differs in its larger, 4-merous flowers with petals longer than wide.

—BEN LEGLER AND DAVID GIBLIN, WTU Herbarium, Burke Museum, Box 355325, University of Washington, Seattle, WA 98195-5325; blegler@u.washington.edu.

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NOTEWORTHY COLLECTION

WASHINGTON

BLITUM HASTATUM Rydb. (AMARANTHACEAE). — Okanogan County, Okanogan National Forest, Daisy Campground on north side of Forest Road 39 (Toats Coulee Road), elev. 1487 m, 48.86763° N, 119.861087° W, 3 July 2015, *B. Legler* 13697 (ID, MO, UBC, WTU).

Previous knowledge. *Blitum hastatum* (= *Chenopodium capitatum* [L.] Ambrosi var. *parvicapitatum* S.L. Welsh) is native to the western United States, occurring from northeast Oregon east to southwest Montana and south to California and New Mexico (Clemants and Mosyakin 2003). Disjunct populations have been reported from southern British Columbia just north of the United States border (CPNWH 2015), although it is unknown if these are native or introduced. *Blitum hastatum* differs from the similar *B. capitatum* L. in its smaller (mostly < 5 mm diameter) fruiting glomerules, greenish sepals that do not become fleshy, and cuneate to truncate leaf bases.

Significance. First collection of this species for Washington. Plants at the cited locality were found growing in a campground under mature, open conifer woods in soil disturbed by campers and trampled by cattle, suggesting *B. hastatum* was introduced here.

CORRIGIOLA LITTORALIS L. subsp. *LITTORALIS* (CARYOPHYLLACEAE). — Cowlitz County, Merrill Lake, area from boat ramp north for approximately 0.5 km, elev. 445 m, 46.09384° N, 122.32014° W, 27 August 2013, *Giblin* 5085 (WTU).

Previous knowledge. Strapwort, an herbaceous annual, is an uncommon Eurasian introduction documented in North America from Maryland, Oregon (near Portland), and southwest British Columbia (Thieret and Rabeler 2005, CPNWH 2015).

Significance. Not previously documented in Washington. The population at this location was extensive, with thousands of small plants forming scattered mats along the mucky shoreline in the drawdown zone of the reservoir. It is to be expected in similar habitats in reservoirs downstream from this site.

CYPERUS FUSCUS L. (CYPERACEAE). — Clark County, east shore of Columbia River ca 0.8 km south of Frenchmans Bar Regional Park, elev. 3 m, 45.673203° N, 122.764733° W, 11 September 2015, *B. Legler* 13841 (MICH, WTU).

Previous knowledge. Brown galingale is native to Eurasia, with introduced populations occurring in

scattered areas of North America, including Ontario, Quebec, the New England states, South Dakota, Nebraska, Missouri, Nevada, and California (Tucker et al. 2002).

Significance. First collection for Washington. Plants at this location were locally common on a silty, tidally inundated river shoreline, growing with four other *Cyperus* L. species. Peter F. Zika, Cyperaceae expert, confirmed the specimen identification.

GALEOPSIS BIFIDA Boenn. (LAMIACEAE). — King County, no locality given, August 1949, *Mrs. Newsome s.n.* (WTU). Kittitas County, along John Wayne Trail about 1/2 mile northwest of trailhead south of Thorp, elev. 527 m, T18N R17E S11, 48.07436° N, 120.6360° W, 28 July 2011, *Knoke* 2203 (WTU).

Previous knowledge. Split-lip hemp-nettle is native to a large swath of temperate Eurasia (Afonin et al. 2008), and is introduced to northeastern North America, the midwestern United States, and parts of Canada including British Columbia (Kartesz 2015). *Galeopsis bifida* is sometimes treated as a variety of the similar *G. tetrahit* L.; however, molecular results indicate each is an allopolyploid with different maternal parentage and should be recognized as distinct species (Bendiksby et al. 2011). *Galeopsis bifida* can be distinguished by the emarginate, often revolute-margined, lower corolla lobe with more extensive dark coloration.

Significance. Not previously documented for Washington. The cited collections were previously misidentified as *G. tetrahit*.

HIRSCHFELDIA INCANA (L.) Lagrèze-Fossat (BRASSICACEAE). — Clark County, NE 159th Street at railroad crossing just east of junction with NE 137th Ave, south of Battle Ground, elev. 87 m, 45.737319° N, 122.531088° W, 11 September 2015, *B. Legler* 13861 (NY, RM, WTU); Cowlitz County, shoulder of Meeker Drive just north of Kingwood Street, on east side of Interstate 5 in Kalama, elev. 10 m, 47.739336° N, 122.30662° W, 17 June 2015, *B. Legler* 13612 (COLO, HPSU, WTU); Jefferson County, along Larry Scott Trail ca 1.1 km southwest of trailhead at marina, south side of Port Townsend, elev. 2 m, 48.098377° N, 122.791641° W, 13 May 2016, *B. Legler* 14064 (WTU); King County, northwest side of Shorecrest High School in Shoreline, slope between parking lot and sports fields, elev. 123 m, 47.741921° N, 122.304944° W, 30 May 2015, *B. Legler* 13565 (GH, SRP, WTU); same site, 18 Jul 2015, *B. Legler* 13764 (HPSU, MICH, UCR, WS, WTU); Diagonal Avenue South Access for Duwam-

ish River, elev. 2 m, 47.56025° N, 122.34418° W, 8 June 2015, *Giblin 5601* (WTU); shoulder of Des Moines Memorial Drive South just south of on-ramp for Highway 518, elev. 79 m, 47.468723° N, 122.320124° W, 26 August 2015, *B. Legler 13809* (ARIZ, CHSC, IDS, WTU); northeast side of Boeing Ponds, 62nd Avenue South, Kent, elev. 60 m, 47.42164° N, 122.25712° W, 15 November 2015, *Giblin 5822* (WTU); West Point, on west side of Discovery Park, Seattle, elev. 2 m, 47.661098° N, 122.433521° W, 23 June 2016, *S. Legler 2016-01* (WTU); along Burke-Gilman Trail 120 m northwest of junction with 30th Avenue NE, near University Village, Seattle, elev. 18 m, 47.665279° N, 122.296806° W, 21 August 2016, *B. Legler 14320* (WTU); Pierce County, east side of Bridgeport Way at intersection with Pacific Highway South, Tacoma, elev. 83 m, 47.14883° N, 122.504645° W, 27 September 2015, *B. Legler 13927* (CAS, MO, UBC, WS, WTU); fence line on west side of Interstate 5 ca 230 m south of intersection of Pacific Highway South and 108th Street SW, Tacoma, elev. 86 m, 47.15729° N, 122.487821° W, 27 September 2015, *B. Legler 13928* (SOC, WTU); east side of South Tacoma Way (Pacific Highway) just north of 96th Street South, Tacoma, elev. 88 m, 47.171785° N, 122.48353° W, 27 September 2015, *B. Legler 13929* (MO, WTU); South 26th Street just east of intersection with Pacific Avenue, Tacoma, elev. 25 m, 47.238305° N, 122.434213° W, 27 September 2015, *B. Legler 13931* (WTU); Milwaukee Way just south of where it passes under Lincoln Avenue, in Tacoma port area, elev. 4 m, 47.255449° N, 122.404863° W, 27 September 2015, *B. Legler 13932* (WTU); Snohomish County, west side of Mountain Loop Highway 200 m north of East Alpine Street, Granite Falls, elev. 120 m, 48.088697° N, 121.963462° W, 7 September 2015, *B. Legler 13832* (MONTU, WTU); south side of Port Gardner Way just west of West Marine View Drive, Everett, elev. 6 m, 48.000109° N, 122.214611° W, 17 October 2015, *B. Legler 13946* (CAS, UBC, WTU, WWB); Whatcom County, east side of Squalicum Creek Park, Bellingham, elev. 13 m, 48.768409° N, 122.499375° W, 6 September 2015, *B. Legler 13824* (UBC, WTU, WWB).

Previous knowledge. Mediterranean mustard is native to the Mediterranean region of Eurasia and northern Africa, with North American introduced populations known in California, Idaho, Nevada, Oregon, Arizona, and Mexico (Warwick 2010; CPNWH 2015). It is an invasive weed in California (California Invasive Plant Council, <http://www.cal-ipc.org/>). In Oregon it is known from only a few recent and older collections, with the oldest specimen collected on the ballast grounds at Linnton, Oregon, in 1919 (CPNWH 2015).

Significance. First collections for Washington. The 18 specimens cited above span the length of western Washington from Whatcom County near the Canadian border south through the Puget Sound

lowlands to Clark County just north of Oregon. Additional plants, not collected, were observed at multiple locations on the margins of Interstate 5, Interstate 90, and Highway 2 through much of the central and southern Puget Sound region. The large number of locations and widespread distribution is somewhat surprising, as it is unlikely that such a large plant would have gone undetected for very long. We suspect that *Hirschfeldia incana* may be a recent and rapidly spreading arrival in Washington. However, plants may have been overlooked as *Brassica nigra* (L.) W.D.J. Koch, a widespread and similar species with silique valves prominently 1-veined and beak seedless (vs. valves obscurely veined and beak 1-seeded in *H. incana*). At most of the cited localities the species grows in dry, gravelly or loamy soil of roadsides, embankments, recent construction sites, vacant lots, and recently seeded lawns. Flowers were observed from early May through mid November, and mature, indehiscent fruits from June through the middle of November. Plants were observed to produce large numbers of seed-bearing fruits, with seeds likely spread by vehicles and construction equipment.

LACTUCA VIROSA L. (ASTERACEAE). — King County, University of Washington campus, on southeast side of Ocean Sciences Building adjacent to NE Boat Street, elev. 11 m, 47.650951° N, 122.312606° W, 4 October 2015, *B. Legler 13936* (WTU); south side of Hitchcock Hall on University of Washington campus, Seattle, elev. 16 m, 47.651694° N, 122.312002° W, 10 August 2016, *B. Legler 14276* (NY, WTU); Lewis County, access area on shore of Riffe Lake along Champion Haul Road 3.4 air km southwest of Glenoma, elev. 212 m, 46.488584° N, 122.182829° W, 23 September 2014, *B. Legler 13381* (WTU); same site, 13 Sep 2015, *B. Legler 13913* (MO, UBC, WTU); junction of Glenoma Road and Champion Haul Road, near northeast end of Riffe Lake, elev. 240 m, 46.50139° N, 122.1803° W, 13 September 2015, *B. Legler 13914* (CAS, WTU); shoulder of Highway 12 at paved pull off overlooking Riffe Lake, 8.5 air km WSW of Morton, elev. 323 m, 46.534582° N, 122.380891° W, 13 September 2015, *B. Legler 13915* (ID, WTU).

Previous knowledge. Bitter lettuce is native to Europe, with introduced populations known from Alabama, California, and Washington D.C. (Strother 2006). No previous records are known from the Pacific Northwest; an old specimen misidentified as *L. virosa* from Park County, Montana (*Hitchcock 13573*, WTU) is *L. serriola* L. Fruits of *L. virosa* transition from lemon-yellow through orange to brick red when young, becoming deep purplish-black at maturity. In contrast, fruits of the similar and widespread *L. serriola* are whitish or tan when young and light to medium brown at maturity. The two species also differ in fruit shape, margins, and apical serrations.

Significance. First collections for Washington. The species appears to be well established on roadsides near Riffe Lake, Lewis Co., and should be sought elsewhere in western Washington.

LUPINUS PACHYLOBUS Greene. (FABACEAE). — San Juan County, Sentinel Island, west end of island, elev. below 50 m, 48.6400° N, 123.1523° W, 27 April 2006, *Habegger EH-1060* (WTU).

Previous knowledge. Big pod lupine is otherwise native and endemic to California (Baldwin et al. 2012).

Significance. First collection for Washington and first collection outside of California. The specimen label stated plants were found in “several small patches on dry south slope” of the island. Although several other presumably native species are disjunct between the San Juan Islands and California (e.g., *Crassula connata* [Ruiz & Pav.] A. Berger and *Lepidium oxycarpum* Torr. & A. Gray), we suspect *Lupinus pachylobus* is introduced. A possible point of introduction is nearby Speiden Island, on which exotic game animals are raised and the slopes are heavily grazed. *Lupinus pachylobus* differs from the widespread *L. bicolor* Lindl. in its wider pods (7–9 mm wide vs. 3–6 mm wide) and glabrous keel petals; the cited specimen has both flowers and mature dehiscing pods, and was previously misidentified as *L. bicolor*.

OXYBASIS GLAUCA (L.) S. Fuentes, Uotila & Borsch subsp. *GLAUCA* (AMARANTHACEAE). — Franklin County, Wahluke Wildlife Parcel on east side of Mountain Vista Road, approximately 1 km south of Michel Road, elev. 282 m, 46.66375° N, 119.30076° W, 17 September 2015, *Giblin 5742* (WTU).

Previous knowledge. *Oxybasis glauca* subsp. *glauca* (= *Chenopodium glaucum* L. var. *glaucum*) is a European introduction, widespread in central and eastern North America, occurring west of the Rocky Mountains in Nevada (Clemants and Mosyakin 2003) and southwest British Columbia (CPNWH 2015). Baldwin et al. 2012 lists it as “expected” in California. Subspecies *glauca* is distinguished from North America native *O. glauca* subsp. *salina* (Standl.) Mosyakin by its smaller seeds (0.6–0.9 mm vs. 0.9–1.1 mm) and inflorescences lacking leafy bracts distally.

Significance. Not previously documented from Washington.

VICIA LUTEA L. (FABACEAE). — Jefferson County, Port Townsend, west side of Larry Scott Memorial Trail about 100 m south of milepost 1.0, elev. 2 m, 48.097247° N, 122.793846° W (coordinates obtained from the collector in 2015), 13 May 2007, *Weinmann 345* (WTU); same general site, 48.098377° N, 122.791641° W, 13 May 2016, *B. Legler 14063* (UBC, WTU).

Previous knowledge. Yellow vetch is native to Europe, and introduced to North America at scattered localities in California, western Oregon, and the southeastern United States (Kartesz 2015).

Significance. First collections for Washington. Plants at the cited locality are locally common and well established as a weed on a long stretch of dry trailside bank. The first cited specimen was previously misidentified as *Vicia pannonica* Crantz, a similar yellow-flowered species with pubescent banner petals and pod hairs not pustulate-based.

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SOIL CHEMISTRY PATTERNS IN AN EDAPHIC ENDEMISM HOTSPOT: THE PEBBLE PLAINS OF THE SAN BERNARDINO MOUNTAINS, CALIFORNIA

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ABSTRACT

Pebble plains are a unique edaphic environment known only from the high valleys of the San Bernardino Mountains, California. The pebble plains have long been celebrated for the high vascular plant diversity they support, including at least six taxa endemic to the San Bernardino Mountains. Past research has shown that pebble plains soils differ most notably from nearby non-pebble plains soils in terms of their high clay content and loose, stony structure, especially in their upper-most horizon and on the soil surface. The stony upper horizon is probably the result of frost-heaving and erosion of soil particles by wind; the resulting accumulation of stone fragments at the soil surface is what inspired the name “pebble plains.” The combined effects of a friable, rocky surface, heavy lower horizons, frost heaving, high solar insolation, and desiccating winds are thought to limit recruitment of shrubs and trees and foster the persistence of a unique pebble plains flora consisting of herbaceous annuals and low-growing perennials. Despite decades of research involving the pebble plains and their unique flora, the soil chemical properties of pebble plains versus surrounding (non-pebble plains) soils has not been thoroughly investigated. This study investigates the chemistry of pebble plains soils to determine if they are chemically divergent from adjacent non-pebble plains soils. To answer this question, we collected soils from nine pebble plains areas, sampling from both the pebble plains themselves and from surrounding, non-pebble plain forests or shrublands. These samples were subjected to analyses for 13 soil chemical properties. Multivariate analyses of these data indicate that habitat type (pebble plains versus non-pebble plains) is the single most important factor explaining the variation in soil chemical properties. Although only Zn concentration is significantly divergent between the habitat types, pebble plains soils are generally deficient in major- and micronutrients compared to adjacent non-pebble plains soils. Our results suggest that while physical factors such as frost heave may be the primary agents responsible for the original formation and persistence of the pebble plains flora, the soils of the pebble plains are chemically unique, which may reinforce physical constraints on floristic composition in these areas.

Key Words: edaphic endemism, ecology, rare plant, Transverse Ranges.

Plant-soil interactions play a key role in plant evolution and ecology, influencing local adaptation, speciation, and the formation of plant communities (Kruckeberg 1986, 2002; Rajakaruna 2004). Although it is easiest to think of soils as influencing plant ecology and evolution via completely abiotic effects, especially in the case of extremely chemically stringent soils like serpentine (Kruckeberg 1986), it is also well known that plant-soil interactions are a primary driver of soil formation and so the influence of soils on plants is often mediated by the plants themselves (reviewed in Ehrenfeld et al. 2005).

Recent research, especially on model soils like serpentine (Harrison and Rajakaruna 2011), has helped scientists to gain a nuanced perspective on how soils affect plant evolution, community assembly, and regional diversity (Anacker et al. 2011; Damschen et al. 2012; Anacker and Harrison 2012; Cacho and Strauss 2014; Burge and Salk 2014; Baldwin 2014; Anacker 2014). Nevertheless, only a handful of unusual soil systems other than serpentine have been examined in detail with respect to their physical and chemical properties and the influence of these properties on plant life (Moore et al. 2014;

Saslis-Lagoudakis et al. 2014), preventing inference of general patterns concerning the role of soils in plant evolution and ecology. Here, we focus on the pebble plains of the San Bernardino Mountains, California. A pebble plain is a distinctively open, alpine-like, dwarf shrub- and herb-dominated habitat restricted to moderate elevations (1800–2300 m) primarily on the northeast side of the San Bernardino Mountains (Derby and Wilson 1978, 1979; Krantz 1994). Pebble plains are extremely limited in area, with less than 221 ha extant (U.S. Department of the Interior 2006). Pebble plains are found on flats, gentle slopes, and mesas in Bear Valley and Holcomb Valley, with only a handful of outlying occurrences (Fig. 1; Derby and Wilson 1979; Ciano 1984; Neel and Barrows 1990; Krantz 1994; U.S. Department of the Interior 2006).

Pebble plains have long been of interest to botanists due to the diverse and seemingly specialized flora that they support; at least 53 native vascular plant minimum rank taxa (MRT; species, subspecies, and varieties) are found in the pebble plains habitat, 27 of which (51%) have a high level of affinity for this habitat type in the San Bernardino Mountains

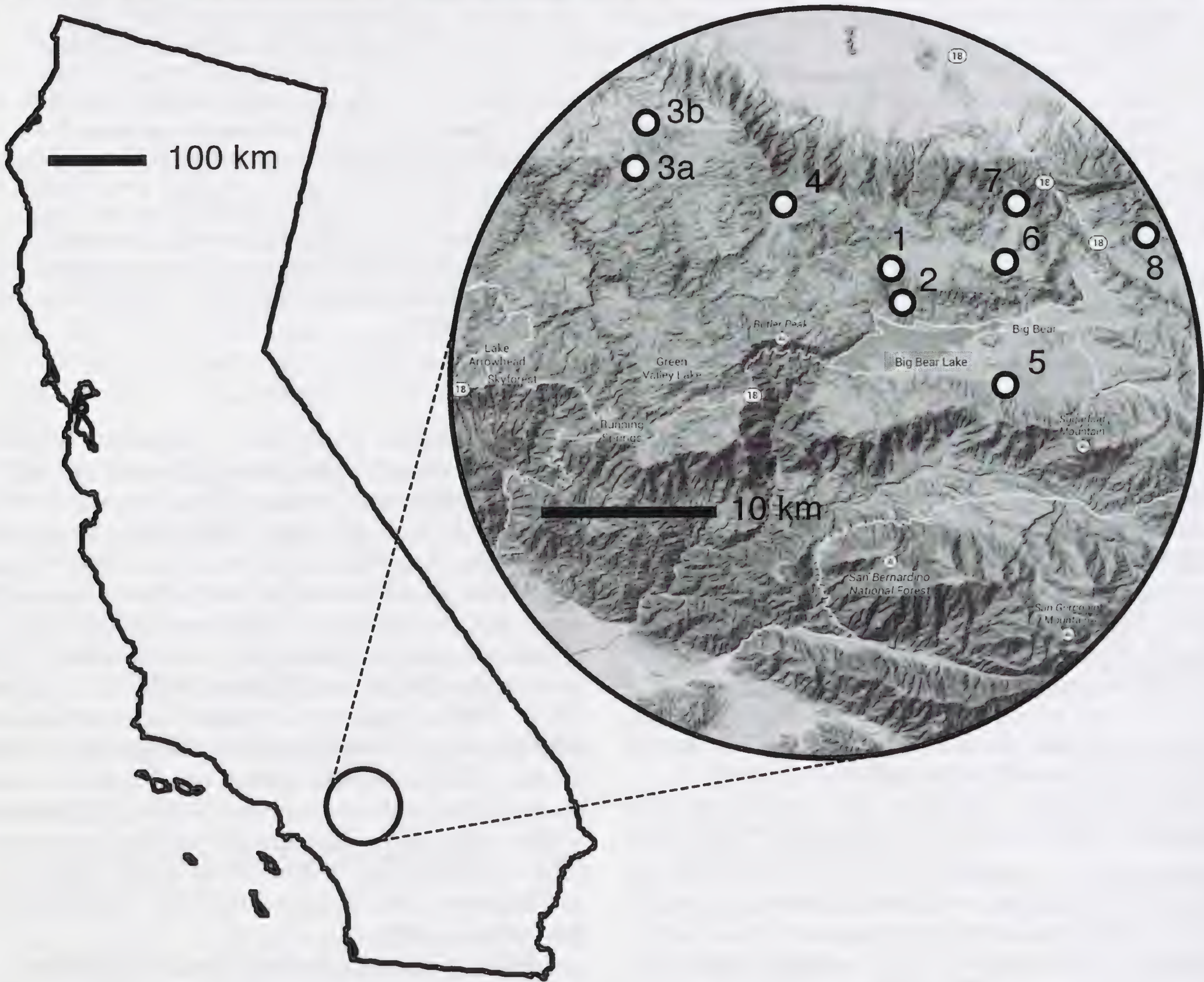
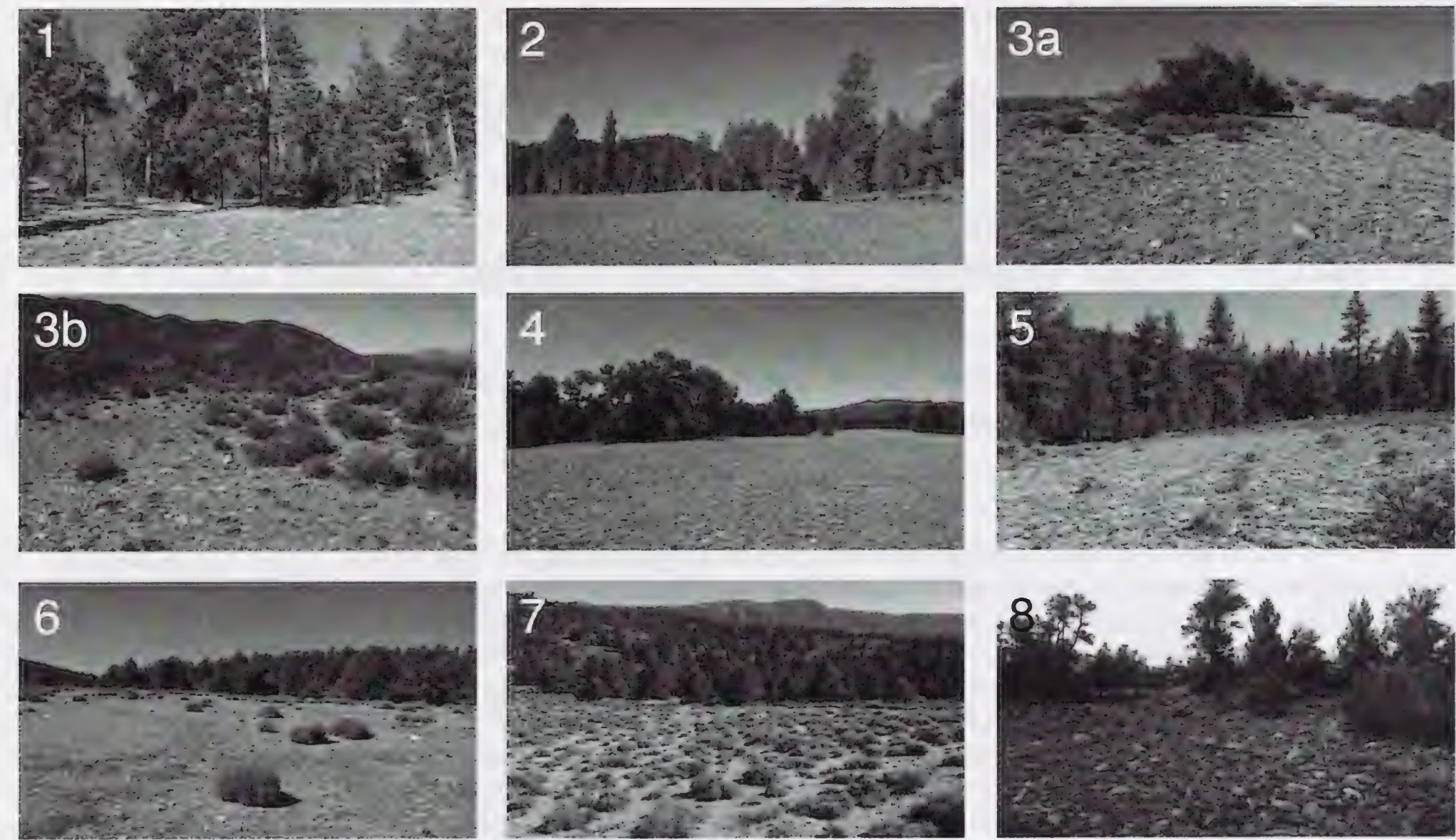


FIG. 1. Sampling map and site photos. Field sites (Table 1) indicated by circles on map of the San Bernardino Mountains. Map data for San Bernardino Mountains: Google.

(Appendix 1). These taxa are strong indicators of pebble plains habitats (Derby and Wilson 1978, 1979) but are occasionally found on carbonates or in vernal wetlands where heavy clay soils exist (Krantz 1994; Stoughton unpublished). This suite of specialists includes six MRT that are endemic to the San Bernardino Mountains and are predominantly found in pebble plains: *Boechera parishii* (S. Watson) Al-Shehbaz, *Castilleja cinerea* A. Gray, *Dudleya abramsii* Rose subsp. *affinis* K.M. Nakai, *Eremogone ursina* (B.L. Rob.) Ikonn., *Ivesia argyrocoma* (Rydb.) Rydb. var. *argyrocoma*, and *Linanthus killipii* H. Mason (Derby and Wilson 1978, 1979; Krantz 1983, 1994; Ciano 1984; U.S. Department of the Interior 2007). The suite of 27 specialists with high level of affinity for pebble plains (Appendix 1) also contains plants that may represent taxa new to science, including *Androsace elongata* L. aff. subsp. *acuta* (Greene) G.T. Robbins, *Echinocereus* sp. aff. *engelmannii* (Engelm.) Lem., *Gilia* sp. aff. *diegensis* (Munz) A.D. Grant & V.E. Grant, and *Primula* sp. aff. *hendersonii* (A. Gray) Mast & Reveal (T. Stoughton, unpublished data). Two of the pebble-plains associated MRT, *Eremogone ursina* and *Echinocereus* sp. aff. *engelmannii*, are apparently strict pebble plains endemics that occur nowhere else (Appendix 1).

Pebble plains soils overlay a variety of rock types, and are influenced to some degree by these host rocks. However, the unique soils of the pebble plains are probably not derived from geological parent materials found in the San Bernardino Mountains; instead, the soils of the pebble plains probably have a more ancient origin, in the Tertiary plains that predate uplift of the San Bernardino Mountains around 3 Ma (Krantz 1994; Sadler and Reeder 1983). Evidence of this origin is found in the pebbles that give the pebble plains their name, which are nearly invariably composed of quartzite, a geological parent material that is rare in the San Bernardino Mountains. Instead of originating in a montane environment, the pebble plains soils likely began as desert pavements, and were carried to their current elevations by uplift of the San Bernardino Mountains (Sadler and Reeder 1983). Today, these soils are widespread in the San Bernardino Mountains, underlying forests and shrublands as well as the floristically distinctive pebble plains (Krantz 1994). Despite a common origin, only some of these soils have given rise to pebble plains, and it is not yet clear what drove differentiation initially, or what maintains it, but it has been proposed by Krantz (1994) that their persistence in low slope-angle situations suggests a relationship with reduced erosion of the fine clays that (in part) make up pebble plains soils.

Pebble plains soils differ strongly from nearby non-pebble plains soils in having a higher clay content as well as a distinctive structure, with a loose, friable, pebble-rich (primarily quartzite) layer near the surface (Derby and Wilson 1978, 1979; Derby 1979 [tabulating unpublished data of M. Lund]; Krantz 1994; U.S. Department of the Interior

2006). The distinctive structure of pebble plains soils is probably influenced by frost-heaving during freeze-thaw cycles of the winter and early spring, which cyclically disrupt the soil surface, allowing wind to carry away fine soil particles, leading to the accumulation of stone fragments (predominantly quartzite pebbles) at the surface (Derby and Wilson 1978; Derby 1979; Derby and Wilson 1979; Krantz 1994; U.S. Department of the Interior 2006). Overall, the combined effects of cyclical frost heaving, heavy clay soil in the lower horizons, high solar insolation, and desiccating winds are thought to limit recruitment of woody perennial plants, especially trees, and foster the persistence of a unique flora consisting of annuals and low-growing perennials. Pebble plains are recognized by their barren aspect, forming variably sized glades within an otherwise forested region (Derby and Wilson 1978, 1979; Ciano 1984; Krantz 1994). Small pebble plains areas may include occasional shrubs or small trees, but more characteristic pebble plains are free of woody perennials with the exception of low-growing sub-shrubs with thick, woody caudices (Krantz 1994).

The plant community of the pebble plains is reminiscent of the isolated alpine flora of San Gorgonio Mountain, which reaches 3506 m, the highest point in the San Bernardino Mountains as well as the Transverse Ranges as a whole (Derby and Wilson 1979; Krantz 1994). However, the flora of the pebble plains occurs up to 1300 m lower than the alpine flora of San Gorgonio Mountain, in the midst of dense montane and sub-alpine forests, including pinyon-juniper woodlands. Derby and Wilson (1979) and Krantz (1994) suggested that the flora of the pebble plains originated during the most recent glacial period, when year-round snow and even small glaciers occurred in the highest elevations of the San Bernardino Mountains (Sharp et al. 1959, reviewed by Krantz [1994]). At this time the alpine flora of San Gorgonio Mountain may have extended down into Bear and Holcomb Valleys, where pebble plains now occur (Krantz 1994). This idea is based on the work of Axelrod (1966) on the Pleistocene (1 Ma) Soboba flora of the San Jacinto area, which showed a more than 1000 m depression of life zones during an apparent glacial period. Krantz (1994, p. 97) provided an eloquent summary of the idea: "During interglacials, the conifer forests climbed to their present levels, except on the clay soils which prevented their establishment and it is on these clay pebble plains that the Pleistocene alpine flora of Big Bear Valley persists to this day, now 1300 m below the alpine summit of Mt. San Gorgonio." Although the alpine flora of San Gorgonio Mountain shares many similarities with the pebble plains (Derby and Wilson 1979; Krantz 1994; Stoughton unpublished), the authors of the present work are not aware of research that has directly addressed the hypothesis of an alpine origin for the pebble plains flora.

Although the pebble plains flora is unique, not least because of its distinctive physiognomy and

unusual distribution at a much lower elevation than similar plant communities, the pebble plains may simply be a well-studied example of a more general phenomenon that occurs in high mountain and high latitude regions, where annual cycles of frost disruption and snow accumulation lead to the formation of distinctive plant communities, some of them at lower elevations than would be expected (Billings and Mooney 1968). Comparative research pursuing this idea would be a worthwhile effort, but is beyond the scope of the present work.

Despite their apparent long-term persistence in the San Bernardino Mountains, there is evidence for gradual degradation of small pebble plains areas, as well as the edges of larger pebble plains, by natural encroachment of forest or woodland vegetation (Derby and Wilson 1979). It is thought that the shade and litter-fall associated with established trees at the edges of pebble plains leads to the recruitment of new tree seedlings and the expansion of the forest habitat into former pebble plains habitat (Derby 1979, from unpublished work of M. Lund). However, the dynamics of this succession-like phenomenon have not been studied in detail as far as the authors know.

The strongly endemic flora of the pebble plains, combined with their very small area, makes them of conservation concern (Neel and Barrows 1990; U.S. Department of the Interior 2006). Though the pebble plains probably never occupied a large area, creation of Big Bear Lake, urbanization of Big Bear Valley, and off-road vehicle activity in San Bernardino National Forest lands have probably reduced the extent of the pebble plains by more than 20% since the arrival of Europeans in the upper elevations of the San Bernardino Mountains in 1845 (Neal and Barrow 1990; Krantz 1994). In addition to threats from urbanization and off-highway vehicle use, the pebble plains now also face the specter of increasing temperatures due to human-caused global climate change as well as increased competition with non-native species, highlighting the urgent need for research to understand the ecology of this unique habitat type and its associated biota before climate change alters it forever.

Even with decades of scientific interest (Ciano 1984; Derby and Wilson 1978, 1979; Krantz 1994) and conservation work (Neel and Barrows 1990; U.S. Department of the Interior 2006, 2007; Parker 2012) involving the pebble plains flora, little is known about how the pebble plains themselves formed and how their unique flora originated, with the exception of a few hypotheses brought forward by Krantz (1994), as described above. To our knowledge, no ecological studies have attempted to experimentally address the question of why certain species are associated with the pebble plains, or how the pebble plains endemic taxa evolved. For research and conservation purposes, it is important to learn how the pebble plains soils differ from adjacent non-pebble plains soils, and what properties allow them

to support a unique collective flora. In addition to providing a better understanding of how edaphic endemism evolved in the pebble plains and how the pebble plains persist as an edaphic phenomenon, such work would lead directly to positive conservation outcomes by suggesting criteria for assessing the conservation value of pebble plains sites, and appropriate ex-situ conservation conditions for rare plants.

The present work originated during a series of discussions between the two lead authors on the problem of what edaphic endemism is and how it evolves. Because of their insular or outcrop-like geographic distribution (Ciano 1984; Krantz 1994), the pebble plains present an interesting parallel to better studied systems in which edaphic endemic plants are restricted to soils with a particular geological origin (e.g., serpentine soils derived from the erosion of serpentinite). We agreed to take a closer look at the pebble plains to examine more closely what makes them unique. The aim of our work was to find out whether pebble plains soils are consistently chemically divergent from adjacent non-pebble plains soils, similar to the way that serpentine soils differ from adjacent non-serpentines in terms of their fertility, Ca to Mg ratio, and heavy metals content (Kruckeberg 1986). To test this, we collected soils from nine pebble plains sites in the San Bernardino Mountains, sampling from the pebble plains themselves and from surrounding, non-pebble plain forests or shrublands. These samples were subjected to chemical analysis, with data obtained for 13 soil chemical properties including pH, conductivity, nitrate, and a suite of ten major- and micronutrients. Multivariate analyses of these data were carried out to summarize relationships among soils, determine soil properties associated with pebble plains versus non-pebble plains, and determine whether pebble plains soils are consistently chemically divergent from non-pebble plains soils.

MATERIALS AND METHODS

Assessing Parent Material

Surface-born pebbles were obtained from each of the nine sites, sampling haphazardly from the areas where soil samples were obtained; 1/2 kg of pebbles were obtained from each site. Pebbles were examined by T. S. to determine their geological origin using standard physical property tests (hardness, color, streak, luster, cleavage, and chemical reaction).

Soil Sampling Methods

On 2 and 3 August 2015 nine pebble plains sites were visited with the aim of representing all major areas of pebble plain distribution (Fig. 1; Table 1). At each site, two soil samples were collected, one from pebble plain habitat and one from adjacent scrubland or forest without the characteristic soil structure

TABLE 1. Soil sampling localities. Latitude and longitude reported in WGS 84 datum, accurate to 5 m or less; elevation from GPS at three-dimensional accuracy of 5 m or less.

Site	Latitude	Longitude	Elevation (m)
1	34.2876	−116.9196	2270
2	34.3068	−116.9279	2200
3a	34.3396	−117.0648	1784
3b	34.337	−117.0654	1767
4	34.2437	−116.8507	2178
5	34.305	−116.8501	2273
6	34.2994	−116.821	2088
7	34.2915	−116.8034	2097
8	34.2183	−116.7148	2399

and plant community of the pebble plains. At each site, the pebble plains and non-pebble plains samples were prepared by bulking five sub-samples. Sites for soil sub-sample collection were selected with the aim of representing the slope and aspect diversity of the site as well as varying distances from the interface between pebble plains and non-pebble plains habitats. Sub-samples were located not more than 100 m from one another. Sub-samples were collected using a garden trowel with a steel blade, excavating to a depth of 15 cm. After consolidation, the sub-samples were mixed thoroughly in a 19 L plastic pail, and a 0.5 L composite sample removed for chemical analysis.

Soil Chemistry Analyses

Soil chemistry analyses were carried out by the Texas A & M University Soil, Water, and Forage Testing Laboratory, and are identical to those used by Burge and Manos (2011). Samples were passed through a 2 mm sieve prior to analysis to remove stony fragments. Major nutrients (P, K, Ca, Mg, S) and sodium were extracted using the Mehlich III extractant (Mehlich 1978, 1984) and determined by inductively coupled plasma mass-spectroscopy (ICP).

Micronutrients (Cu, Fe, Mn, and Zn) were extracted using a modified DTPA solution (Lindsay and Norvell 1978), and determined by ICP. Soil pH was determined in a 1:2 soil:deionized water extract (Schofield and Taylor 1955). Electrical conductivity (a proxy for soluble salts) was determined in a 1:2 soil : deionized water extract using a soil conductivity probe (Rhoades 1982). Finally, nitrate (NO₃[−]) was extracted in 1 M KCL solution, reduced to nitrite (NO₂[−]) using a cadmium column, and determined by spectrophotometer (Keeney and Nelson 1982). In total, 13 soil chemical properties were assayed (Table 2).

Statistical Analysis of Soil Chemistry Data

We treated the soil chemistry data in a multivariate statistical framework, visualizing the data and testing for differences between pebble plains and non-pebble plains soils using principal components analysis (PCA), analysis of similarity (ANOSIM), and t-tests. Analyses were done in R, version 3.1.2 (R Development Core Team 2015). Analyses were conducted using scaled data.

PCA was run using default parameters in R. The first two principal components were visualized in bivariate space to examine relationships among sites. The contribution of the soil chemical features to the principal components was determined based on the vector loadings. T-tests were then implemented in R to determine whether the individual soil chemical properties differed between pebble plains and non-pebble plains soils. More complex tests (e.g., via ANOVA) were deemed inappropriate due to the observational nature of the data. A Bonferroni correction for multiple comparisons ($P < 0.00038$) was applied to the results to determine significance.

Analysis of similarity (Clarke 1993) was used to test for overall chemical divergence between pebble plains and non-pebble plains soils. The analysis was carried out using the R package *vegan*, v. 3.2-4 (Oksanen et al. 2016). We used 1000 permutations

TABLE 2. Soil chemistry summary statistics. All statistics reported as average ± standard deviation. Conductivity (Con.) reported as μmol/cm; nitrate (NO₃) and elemental levels reported as parts per million. The t test results are from two-tailed tests that assume unequal variance; Loading PC1 and Loading PC2 are the loadings on the first two principal components from the PCA analysis. “—” indicates that a variable did not contribute to a particular PC axis.

Variable	Pebble plains	Non-pebble plains	T test	Loading PC1	Loading PC2
pH	6.21 ± 0.64	6.43 ± 0.58	0.4436	−0.307	−0.242
Con.	126 ± 30	150 ± 37	0.1266	−0.441	—
NO ₃	0.62 ± 0.52	2.11 ± 2.66	0.1685	−0.136	0.147
P	35.34 ± 16.16	50.43 ± 22.13	0.07	−0.283	—
K	142.68 ± 51.65	183.78 ± 59.34	0.1249	−0.428	—
Ca	1908.85 ± 1357.94	2365.25 ± 796.62	0.1442	−0.413	—
Mg	284.33 ± 106.63	280.79 ± 155.72	0.8038	−0.308	−0.315
S	5.34 ± 1.68	6.79 ± 2.59	0.2229	−0.187	0.322
Na	4.70 ± 2.39	3.13 ± 1.90	0.2568	−0.125	−0.334
Fe	10.90 ± 4.25	18.08 ± 8.81	0.0615	−0.113	0.427
Zn	0.66 ± 0.26	1.42 ± 0.44	0.0004	−0.245	0.399
Mn	6.49 ± 3.98	11.21 ± 8.14	0.0963	—	0.449
Cu	0.43 ± 0.18	0.37 ± 0.21	0.4067	−0.181	−0.244

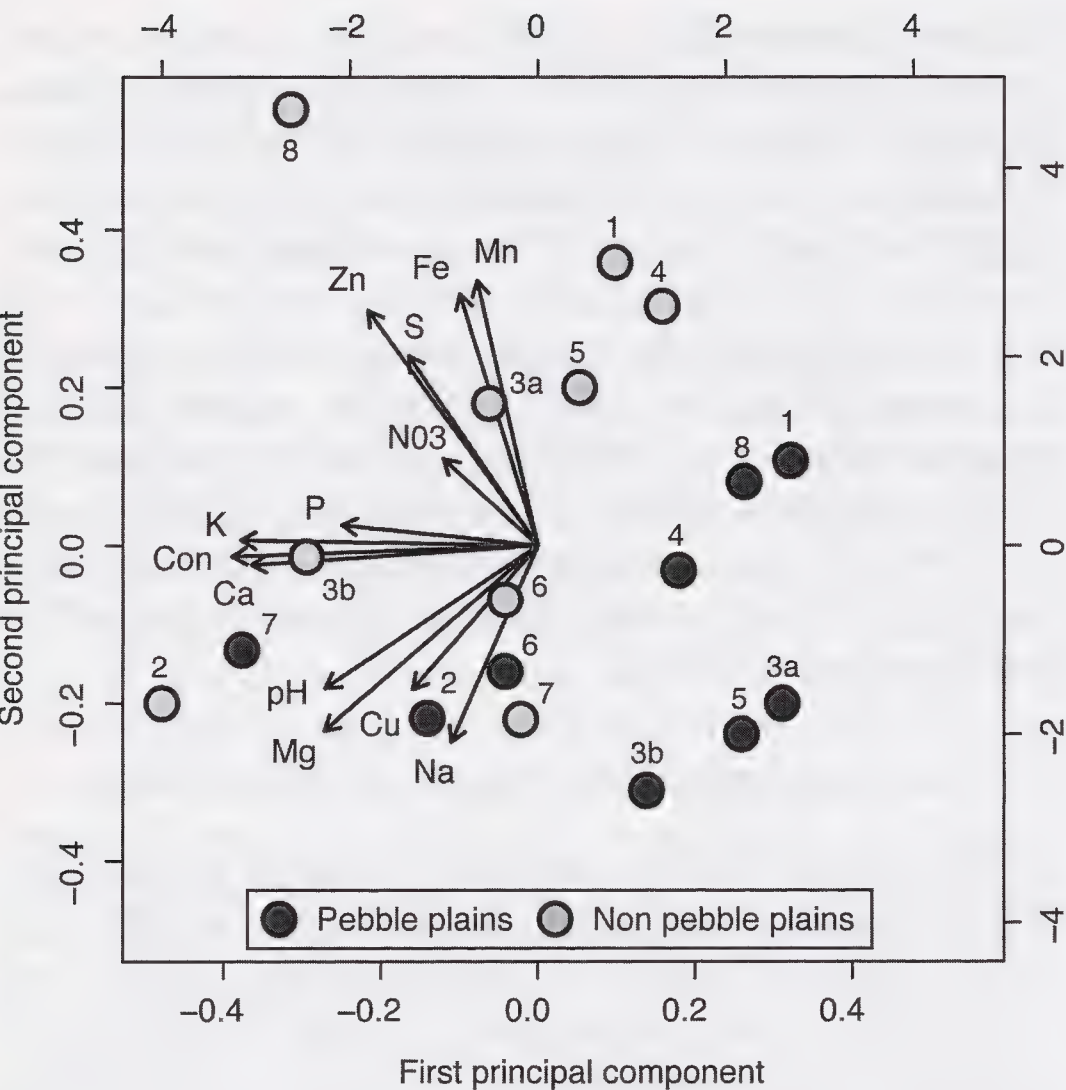


FIG. 2. Plot from principal components analysis (PCA) on soil chemistry. Biplot for first two principal components of PCA for 18 soil samples. Arrows represent direction and magnitude of loading on principal component axes. Symbols: Con = electrical conductivity; NO3 = nitrate.

for the ANOSIM test, with all other parameters at their default settings.

Assessing Plant Community Composition and Relation to Soil

We used multivariate analysis to test for a relationship between soil chemistry and plant community composition of pebble plains sites. All analyses were done in R, version 3.1.2 (R Development Core Team 2015) using the *vegan* package, v. 2.3-4 (Oksanen et al. 2016). To obtain data for these analyses, plant community surveys were carried out for the nine pebble plains sites (Appendix 1). Surveys were carried out by T. S. and D. J., noting all identifiable dead and living taxa present at each site over the same area in which soil samples were obtained; surveys lasted approximately 40 minutes per site.

Lists of taxa for each site (Appendix 1) were translated into a presence/absence matrix, which was used to generate a dissimilarity matrix (Jaccard distances). Only plant taxa found at more than one site were used to generate the dissimilarity matrix. A corresponding dissimilarity matrix was generated for the 13 soil chemical properties (Euclidean distances from scaled data). Community and soil dissimilarity matrices were visualized using hierarchical agglomerative clustering (Faith et al. 1987). The relationship between soil chemistry and plant community composition was then tested using a Mantel test (Legendre and Legendre 1998), comparing the soil matrix to the plant community matrix. For this test

we used the Spearman correlation method, and 999 permutations to assess the significance of the test.

RESULTS

Soil Chemistry and Geological Parent Material

Principal components analysis (Fig. 2) provides the opportunity to visually examine the difference between pebble plains and non-pebble plains soils, as well as the differences between the nine sites. In our PCA, 80% of variance was accounted for by the first three principal components, with 36% of variance in the first principal component (PC), 26% in the second, and 17% in the third. The first PC is most strongly (negatively) correlated with conductivity (vector loading = -0.441 ; Table 2); other elements with strong vector loadings include K (vector loading = -0.428) and Ca (vector loading = -0.413). The second PC is most strongly correlated with Mn (vector loading = 0.449), though many soil chemical features have similarly high loading scores (Table 2), including Fe (vector loading = 0.427) and Zn (vector loading = 0.399). The biplot of the first two principal components (Fig. 2) indicates that pebble plains and non-pebble plains soils are chemically divergent, the divergence being generally associated with lower Zn, Fe, S, Mn, and NO_3 in pebble plains soils (Table 2). After correction for multiple comparisons, t-tests on all 13 variables indicate that only Zn differs significantly between pebble plains and non-pebble plains soils, with lower amounts in pebble plains soils. Analysis of similarity revealed a significant difference in dissimilarity ranks between pebble plains and non-pebble plains soils (ANOSIM R statistic: 0.1636 ; $P = 0.037$; Fig. 4).

Examination of rock samples revealed that pebbles from all nine sites were quartzitic in origin, with few if any differences among sites in terms of qualitative rock composition. Mainly, rock samples collected at each site had a fairly glassy texture and were hard, dense, and non-foliated, being comprised of interlocking grains of quartz.

Plant Community Composition and Correlation with Chemical Data

The composition of the pebble plains plant community varied strongly among sites (Appendix 1). A total of 32 taxa were identified at the nine target sites. Diversity varied between five and 20 taxa, with the highest number at site 8 (20 taxa) and the lowest at site 3b (5 taxa; Appendix 1). Hierarchical clustering demonstrates the plant community relationships among sites (Fig. 4A). In most cases, geographically proximal sites tend to have the most similar community (Fig. 4A). A Mantel test based on dissimilarity matrices for soil chemistry and plant community shows that there is not a significant relationship between soil chemistry and plant community composition ($r = -0.02485$; $P = 0.512$; Fig. 4).

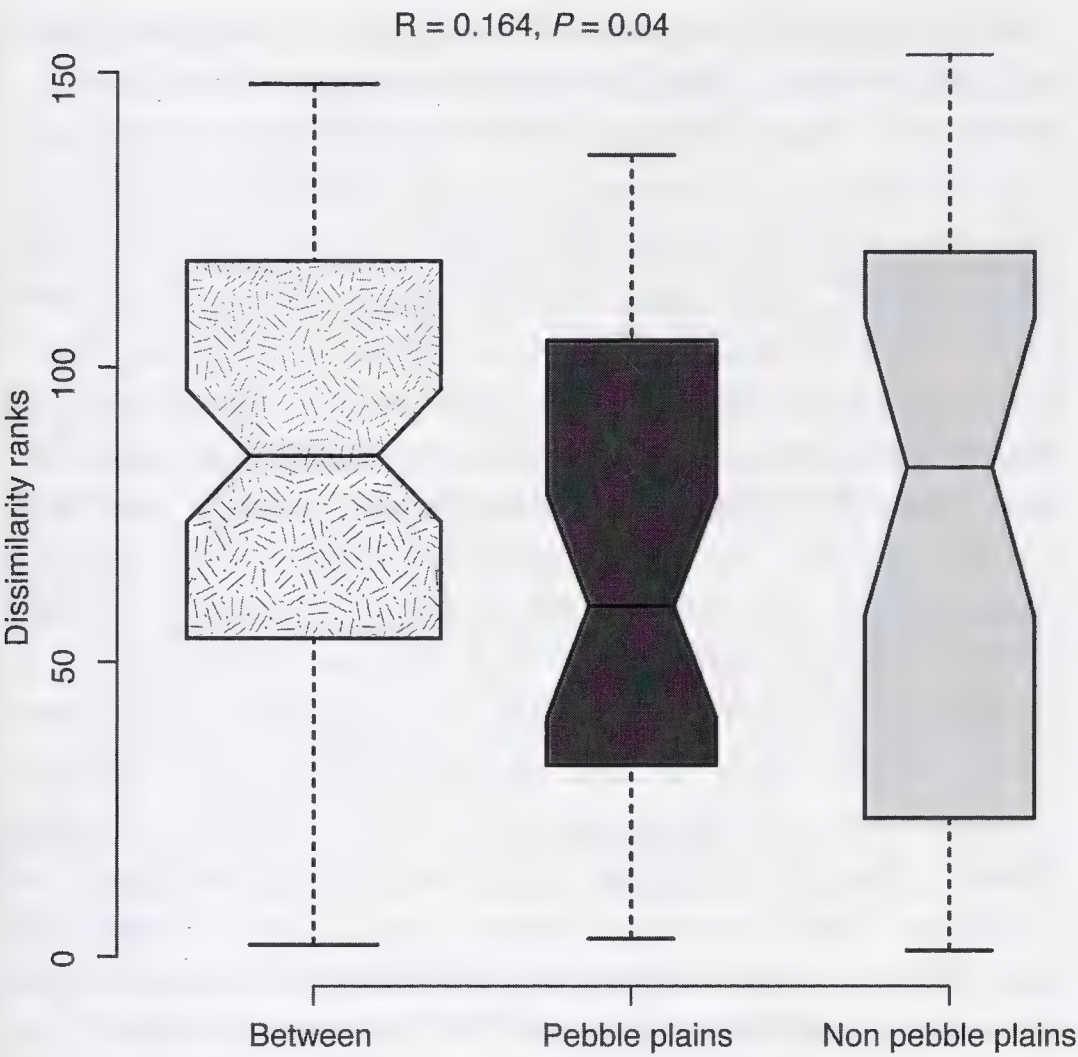


FIG. 3. Results of analysis of similarities (ANOSIM). Results presented as a histogram, with dissimilarity ranks between and within pebble plains and non-pebble plains soils.

DISCUSSION

Pebble Plains Soil Chemistry

Our work suggests that pebble plains sites vary strongly in terms of soil chemistry. However, they are also consistently different from adjacent non-pebble plains soils, with lower levels of major nutrients and micronutrients, particularly Zn. Although our results must be followed up by expanded studies on soil chemistry, as well as studies on physical phenomena that may be linked to pebble plains soil formation

(frost heave, waterlogging, leaching), the results that we obtained corroborate our hypothesis that pebble plains soils are consistently chemically divergent from adjacent non-pebble plains soils, similar to the way that serpentine soils are consistently chemically distinct from non-serpentine soils (Kruckeberg 1986). This result seems to challenge the past assumption that physical conditions are the main factors that differentiate pebble plains soils from non-pebble plains soils (U.S. Department of the Interior 2006). Nevertheless, our results may be consistent with the physical explanation, in that chemical differentiation of pebble plains soils from non-pebble plains soils would be a natural expectation in a physically driven system; exclusion of trees by root-disrupting frost heave, for example, would reduce the amount of nutrients brought to upper soil horizons by decomposition of roots and leaf litter. In addition, exposed conditions in the pebble plains should accelerate leaching and wind-driven loss of soil particles, leading to depletion of vital plant nutrients.

The physical explanation for the persistence of the unique pebble plains plant community is also partly supported by past speculation on the existence of a succession-like phenomenon in pebble plains (Derby 1979; Derby and Wilson 1979), wherein small, isolated pebble plains and the margins of larger pebble plains are converted into forest or woodland by the gradual “creep” of trees and large shrubs into the pebble plain area. As alluded to in the introduction, this process could be driven by the shade and leaf litter cover provided by “nurse” trees at the edges of pebble plains (Derby 1979, citing unpublished data of M. Lund), which are thought to ameliorate the frost heave and extreme insolation experienced in exposed pebble plains, allowing germination and persistence of trees and shrubs (Derby 1979; Derby and Wilson 1979). Though there has been no detailed ecological study of

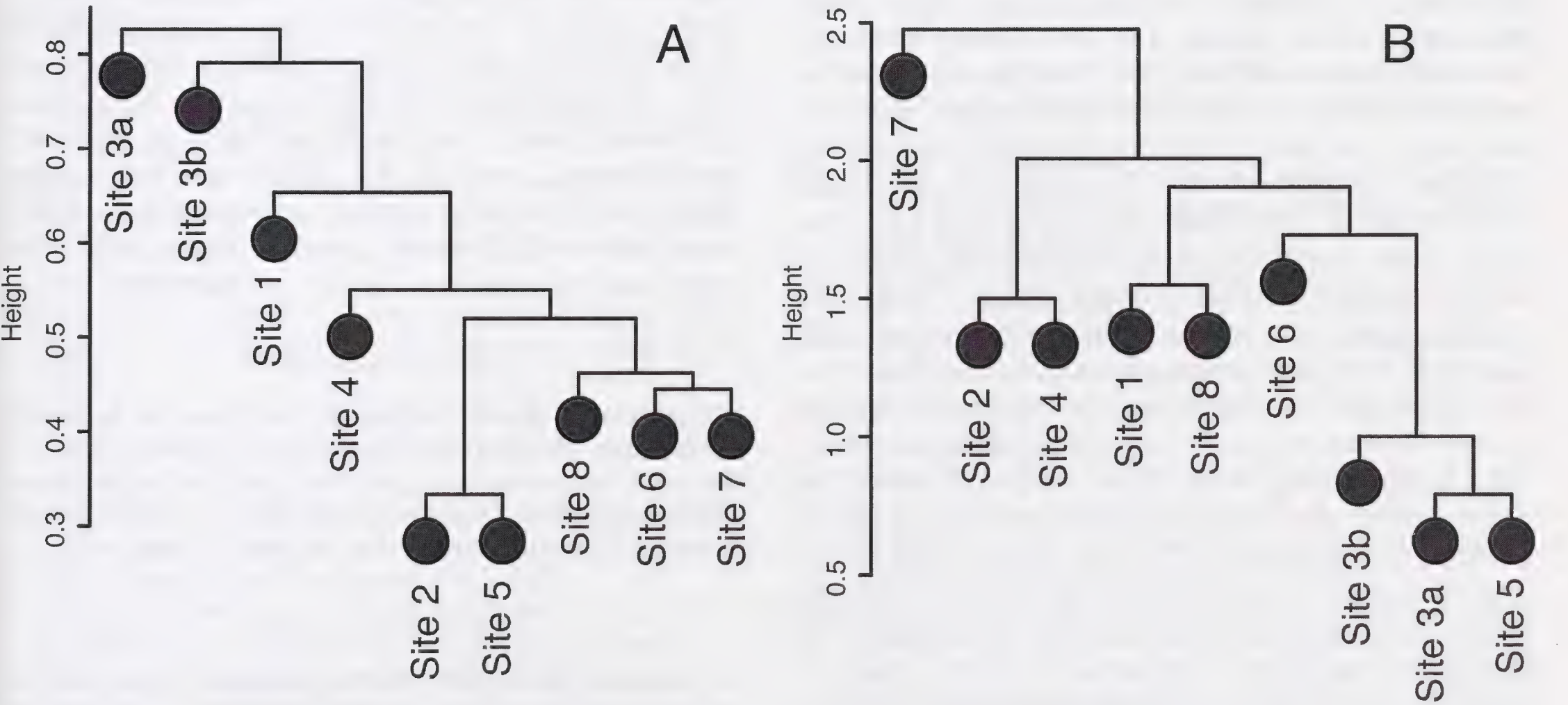


FIG. 4. Results of hierarchical agglomerative clustering based on dissimilarity matrices. A, clustering dendrogram for plant community data. B, clustering dendrogram for soil data.

this succession-like phenomenon other than preliminary studies by Derby (1979, citing unpublished data of M. Lund) on litter accumulation, the idea is consistent with physical rather than chemical factors at work in maintaining the pebble plains, as chemical factors would be expected to repel invasion by trees and shrubs despite the availability of shaded and stabilized sites for seedling germination. However, nutrient redistribution by litter accumulation might provide a chemical explanation. As indicated above, our results should be followed up by experimental work to test whether and how succession occurs in pebble plains.

Overall, our results suggest that while physical processes such as frost heave may be the primary agent responsible for the formation of pebble plains, the resulting soils of the pebble plains are chemically unique, with nutrient status that may be influenced by the feedback of biotic with abiotic processes.

Edaphic Endemism on the Pebble Plains

The pebble plains of the San Bernardino Mountains support a large amount of floristic diversity. This diversity is in the form of a unique suite of dwarfed annual and perennial herbs and subshrubs (~50 MRT; Appendix 1), many of which are strongly associated with pebble plains soils, or completely endemic to them (Derby and Wilson 1978, 1979), and several others which are widespread alpine plants that otherwise grow in the San Bernardino Mountains only around the summit of San Gorgonio Mountain (Krantz 1994). The flora of the pebble plains is reminiscent of floras from other unusual soils—for example serpentines (Kruckeberg 1986), limestone-derived soils (Kruckeberg and Rabinowitz 1985), and gabbro-derived soils (Alexander 2011)—in that they support a locally unusual and strongly endemic flora, and in some cases ameliorate the distributional extension of higher elevation plants into lower elevation habitats (Burge and Salk 2014). However, the pebble plains differ in that they do not appear to be derived from a single geological parent material, and may owe their unusual properties to physical processes instead of the chemical and biological weathering of a chemically unusual parent rock that leads to the formation of most other soils known to support highly edaphic-endemic floras. While our results suggest that pebble plains soils are chemically divergent from non-pebble plains soils, it is likely that this difference is linked to the unusual physical conditions that prevail in the pebble plains, especially frost-heave during freeze-thaw cycles. However, as noted above, the chemical differences that we found could be caused indirectly by frost heave, which would tend to exclude litter-depositing shrubs and trees that would otherwise improve soil fertility by translocating elements from lower horizons to upper ones. In addition, soils that are exposed and disrupted by frost heave would tend to lose nutrients more readily due to leaching and wind erosion.

Pebble plains represent a singular example of an edaphic system, similar to serpentine soil in terms of effect on plant community composition, structure, and endemism. However, much additional research will be needed to determine exactly how pebble plains soils differ from non-pebble plains soils, and how physical, chemical, and biological processes work together to support this unique environment. In particular, it would be desirable to see research that addresses the physical phenomena through observational studies, by looking closely at water content and temperature of pebble plains versus nearby non-pebble plains soils over the course of a year, to determine how freeze cycles, waterlogging, and frost heave might play a role in maintaining the unique structure of pebble plains soils. Experimental studies would also be helpful; for example, transplant or common garden experiments could help determine why woody perennials are not able to establish and thrive on pebble plains, and why certain species are associated with or endemic to pebble plains. The natural complement of such studies would involve experimental removal of tree, shrub, and leaf litter from non-pebble plains to determine whether pebble plains can be “created” by disrupting the climax vegetation type. Additional research is also needed to establish variation in physical properties across a larger number of pebble plains sites. Although such work is beyond the scope of the present study, it is nonetheless critical. As with the chemical study that we carried out, by linking physical information to species lists for each site, it might be possible to determine if there are physical soil properties that support larger amounts of diversity or particular endemics. It would also be possible to correlate the physical information with the chemical information, and thus learn more about how these two factors are interacting in the formation and maintenance of pebble plains. Such research would have clear conservation implications as it would allow for 1) more nuanced design of reserve boundaries to preserve rare plants and the habitats that support them, 2) assessment of habitat quality in the absence of growing plants (many of the pebble plain endemics are ephemeral annuals that might not grow every year), and 3) habitat quality assessments to determine how well “rescue” plants might fare after translocation from one location to another.

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APPENDIX 1. CONTINUED

Family	Taxon	SnBr ¹ endemic	PP endemic ²	PP affinity ³	New taxon ⁴	Endangered ⁵	1	2	3a	3b	4	5	6	7	8
Poaceae	<i>Bouteloua gracilis</i> (Kunth) Griffiths			x				x			x				x
	<i>Elymus elymoides</i> (Raf.) Swezey						x	x		x		x	x	x	x
	<i>Poa secunda</i> J. Presl subsp. <i>secunda</i>						x	x		x	x	x	x	x	
	<i>Stipa hymenoides</i> Roem. & Schult.								x						
	<i>Stipa occidentalis</i> S. Watson var. <i>pubescens</i> (Vasey) J.R. Maze et al.						x				x			x	x
Polemoniaceae	<i>Stipa speciosa</i> Trin. & Rupr.								x						x
	<i>Gilia diegensis</i> (Munz) A.D. Grant & V.E. Grant	x		x	x									x	x
	<i>Leptosiphon breviculus</i> (A. Gray) J.M. Porter & L.A. Johnson						x								
	<i>Linanthus killipii</i> H. Mason	x		x											
Polygonaceae	<i>Microsteris gracilis</i> (Hook.) Greene			x											
	<i>Navarretia breweri</i> (A. Gray) Greene			x											
	<i>Eriogonum kennedyi</i> S. Watson var. <i>austromontanum</i> Munz & I.M. Johnst.			x		x		x	x	x	x	x	x	x	x
	<i>Eriogonum kennedyi</i> S. Watson var. <i>kennedyi</i>			x											
	<i>Eriogonum wrightii</i> Benth. var. <i>subscaposum</i> S. Watson						x		x						x
Primulaceae	<i>Androsace elongata</i> L. subsp. <i>acuta</i> (Greene) G.T. Robbins			x	x										
	<i>Primula hendersonii</i> (A. Gray) Mast & Reveal	x		x											
Rosaceae	<i>Ivesia argyrocoma</i> (Rydb.) Rydb. var. <i>argyrocoma</i>	x		x			x	x			x	x			x
Selaginellaceae	<i>Selaginella watsonii</i> Underw.														
Violaceae	<i>Viola douglasii</i> Steud.			x							x		x		x

APPENDIX 2. Raw soil chemistry data. *Notes.* Conductivity reported as $\mu\text{mol}/\text{cm}$; nitrate and elemental levels reported as parts per million

Site	Habitat	pH	Conductivity	NO ₃	P	K	Ca	Mg	S	Na	Fe	Zn	Mn	Cu
1	Pebble plains	5.26	97	1.57	31.4	111	966.8	145.6	4.93	1.06	14.02	0.59	10.7	0.42
	Non-pebble plains	5.52	120	0.12	44.3	126.4	1975.7	138.9	6.77	2.22	26.56	1.76	16.02	0.31
2	Pebble plains	6.52	147	0.07	41	219.2	2671.3	384	6.1	6.17	11.91	0.93	7.09	0.82
	Non-pebble plains	6.75	224	0.39	49.4	278	4150	641.9	3.89	4.73	20.26	1.43	10.19	0.85
3a	Pebble plains	6.57	96	0.54	26.4	70.3	1119.1	234.9	3.51	3.64	4.35	0.54	2.63	0.23
	Non-pebble plains	6.58	136	6.47	77.5	149.7	2165.3	155.2	7.78	0.82	8.73	1.45	6.08	0.25
3b	Pebble plains	6.44	138	0.29	37.3	115.4	1402.8	427.5	3.31	5.69	5.58	0.41	1.31	0.21
	Non-pebble plains	6.97	185	6.78	96.5	225.4	2690.7	330	6.5	3.6	10.2	1.09	4.74	0.23
4	Pebble plains	5.35	114	0.18	31	104.9	1115.9	253.7	7.36	8.13	16.6	0.67	11.18	0.55
	Non-pebble plains	5.48	125	0.56	40.6	107.6	1356.2	185.7	3.21	1.99	29.04	1.75	14.68	0.24
5	Pebble plains	6.04	111	0.6	18.1	122	1221.9	243.5	3.23	5.2	7.88	0.29	3.29	0.44
	Non-pebble plains	6.37	128	1.05	38.8	143.8	2459.1	164.6	9.09	1.12	19.83	0.95	7.7	0.23
6	Pebble plains	6.67	140	1.39	37.7	183.8	2195.6	383.4	6.34	4.9	10.4	0.91	3.87	0.42
	Non-pebble plains	6.9	132	0.38	44.9	184.5	1943.3	296.5	6.11	3.15	10.8	1.26	5.08	0.42
7	Pebble plains	7.2	189	0.58	73.5	213.1	5200.2	350.4	7.47	6.44	15.32	1.05	6.53	0.46
	Non-pebble plains	6.95	121	0.61	30.5	179.6	1842.3	330.8	5.99	6.9	8.18	0.87	6.38	0.57
8	Pebble plains	5.8	103	0.33	21.9	144.5	1286.1	135.9	5.83	1.1	12.02	0.54	11.78	0.36
	Non-pebble plains	6.36	177	2.62	31.4	259.1	2704.8	283.4	11.79	3.67	29.08	2.22	29.99	0.28

EDAPHIC AND GEOGRAPHIC ORIGINS OF VARIETAL DIFFERENTIATION IN *ERIOGONUM CALCAREUM*

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ABSTRACT

The narrowly endemic vascular plant species *Eriogonum calcareum* (S. Stokes) Grady & Reveal (Polygonaceae) is restricted to barren outcrops composed of Miocene to Pleistocene silicic volcanic ashes scattered throughout southwestern Idaho and southeastern Oregon. We hypothesize that varieties *calcareum* and *sceptrum* are morphologically, geographically, and ecologically differentiated. To test this hypothesis, we measured morphologic and edaphic parameters among 27 populations of *E. calcareum* across the range of the species. *Eriogonum calcareum* var. *calcareum* ranges from eastern Malheur County, Oregon, to southwestern Washington and Payette counties, Idaho, has sessile involucres with hairs sparsely to moderately covering the tube, shorter scape lengths, and narrower leaves. *Eriogonum calcareum* var. *sceptrum* (Reveal) Reveal & Mansfield ranges from Canyon County, Idaho, southeast through Owyhee, Twin Falls, and Elmore counties, has pedunculate involucres that are glabrous to partially floccose at the distal end of the tube, longer scape lengths, and wider leaves. Although some populations have not fully differentiated, plants in the northwestern end of the range (var. *calcareum*) occupy soils significantly higher in both Mg (9.78 cmol/kg; $P < 0.001$) and K (2.84 cmol/kg; $P = 0.018$), and lower in pH (5.15; $P = 0.002$) and Ca/Mg (3.03; $P = 0.016$) than plants in the southeastern end of the range (var. *sceptrum*: Mg: 2.81 cmol/kg; K: 0.82 cmol/kg; pH: 7.82; Ca/Mg: 29.64). The morphological differentiation of the populations justifies a separation of var. *calcareum* and var. *sceptrum* despite the existence of populations with ambiguous morphologies. Population differentiation is likely driven by both geographic isolation and edaphic selection.

Key Words: edaphic, endemism, *Eriogonum*, evolution, exchangeable cations, selection, southeastern Oregon, southwestern Idaho.

Islands offer exceptional opportunities for the study of differentiation and speciation in plants (Stuessy 2006; Comes et al. 2008; Givnish 2010). Barren outcrops comprised primarily of Miocene to Pleistocene lacustrine and fluvial deposits, including beds of silicic volcanic ash, create islands of unique edaphic habitats throughout southeastern Oregon and southwestern Idaho (Swirydczuk 1977; USGS 2015). These islands of barren substrate surrounded by large expanses of shrub-steppe plant communities support many endemic vascular plant species (Table 1).

Despite the large number of species endemic to these diverse edaphic islands, few studies have investigated the relationship between the properties of the soil substrate and either the species' distributions or their evolution (Grimes 1984; Brokaw and Hufford 2010). Edaphic endemism on soils of ultramafic lithologic parentage, in contrast, has been widely investigated (Proctor and Woodell 1975; Kruckeberg 1985; Brooks 1987; Kruckeberg 2002; Brady et al. 2005; Sambatti and Rice 2007; Wright and Stanton 2007; Anacker et al. 2011; Harrison and Rajakaruna 2011; Moore et al. 2014; Escuardo et al. 2015; Medeiros et al. 2015). In particular, cation compositions vary between ultramafic and non-ultramafic soils. In ultramafic soils, unusually high concentrations of Mg, relatively low concentrations of K and Ca, relatively high pH (relative to adjacent soils), low Ca:Mg ratios, and anomalously high

concentrations of other metal cation species, such as Ni, Cr, and Co, create environments that impose intense selection pressures driving adaptation (Sambatti and Rice 2006; Medeiros et al. 2015) and speciation (Brooks 1987; Taylor et al. 2002), and leading to high degrees of endemism (Harrison and Rajakaruna 2011).

The ash deposits that are intercalated throughout the lacustrine and fluvial sediments of Miocene to Pleistocene Lake Idaho and other smaller basins in southeastern Oregon and southwestern Idaho originated in separate volcanic events (Kittleman 1967; Swirydczuk 1977; Kimmel 1979; Izett 1981; Swirydczuk et al. 1982) and are consequently likely to be of differing compositions. Differences in cation composition might be expected in different ash outcrops and such differences might be expected to impose differential selection pressures on the plants inhabiting the barren edaphic outcrop islands of southwestern Idaho and southeastern Oregon. Thus, we investigated the variation among such outcrops in relation to the morphologic variation and geographic distribution in the endemic plant species *Eriogonum calcareum* (S. Stokes) Grady & Reveal.

The regionally endemic *Eriogonum calcareum*, originally described as *E. ochrocephalum* S. Watson subsp. *calcareum* S. Stokes, is restricted to the barren outcrops of the Glens Ferry Formation to the east in Idaho and the Deer Butte and Bully Creek Formations to the west in Oregon. Both as *E.*

TABLE 1. Vascular plant species endemic to silicic volcanic ash outcrops in southwestern Idaho and southeastern Oregon (Grimes 1984; Findley 2004; Mansfield 2010). Distributions and descriptions of Sucker Creek and Bully Creek Formations are from Kittleman (1967); Deer Butte Formation from Corcoran et al. (1962); and Chalk Hills and Glenns Ferry Formations from Malde and Powers (1962), Kimmel (1979), and Swirydczuk et al. (1982). Taxonomy is consistent with Intermountain Flora (Cronquist et al. 1984; Barneby 1989; Cronquist 1994; Cronquist et al. 1997; Holmgren et al. 2005; Holmgren et al. 2012), except when taxa have been described more recently.

Outcrops	Taxa	References
Highly weathered, clay-rich Miocene deposits of the Sucker Creek Formation.	<i>Cymopterus glomeratus</i> Raf. var. <i>greeleyorus</i> (J.W. Grimes & P.L. Packard) R.L. Hartm.	Grimes and Packard 1981, George et al. 2014
	<i>Lomatium bentonitum</i> K.M. Carlson & Mansfield	Carlson et al. 2011
	<i>Lomatium packardiae</i> Cronquist	Cronquist 1992
	<i>Mentzelia mollis</i> M. Peck	Glad 1976
	<i>Phacelia lutea</i> (Hook. & Arn.) J.T. Howell var. <i>calva</i> Cronquist	Halse 1981
Lithified ash-tuff of the Leslie Gulch unit of the Sucker Creek Formation.	<i>Ivesia rhypara</i> Ertter & Reveal var. <i>rhypara</i>	Ertter and Reveal 1977
	<i>Mentzelia packardiae</i> Glad	Glad 1976
	<i>Monardella angustifolia</i> Elvin, Ertter & Mansfield	Elvin et al. 2014
	<i>Phacelia lutea</i> (Hook. & Arn.) J.T. Howell var. <i>mackenzieorum</i> J.W. Grimes & P.L. Packard	Grimes and Packard 1981
	<i>Senecio ertterae</i> T.M. Barkley	Barkley 1981
	<i>Trifolium owyheense</i> Gilkey	Gilkey 1956
Deer Butte, Bully Creek, Chalk Hills and/or Glenns Ferry Formation.	<i>Astragalus cusickii</i> A. Gray var. <i>sterilis</i> (Barneby) Barneby	Cronquist et al. 1989
	<i>Astragalus nudisiliquus</i> A. Nelson	Barneby 1964; Cronquist et al. 1989
	<i>Chaenactis cusickii</i> A. Gray	Cronquist 1994
	<i>Cryptantha propria</i> (A. Nelson & J.F. Macbr.) Payson	Cronquist et al. 1984
	<i>Eriogonum calcareum</i> (S. Stokes) Grady & Reveal	Reveal 1989; Reveal and Mansfield 2014
	<i>Eriogonum chrysops</i> Rydb.	Rydberg 1917
	<i>Eriogonum novonudum</i> M. Peck	Peck 1945
	<i>Eriogonum salicornioides</i> Gand.	Gandoger 1906
	<i>Penstemon miser</i> A. Gray	Gray 1886

ochrocephalum and as *E. calcareum*, two varieties—*calcareum* and *sceptrum*—have been recognized (Reveal 1989; Reveal and Mansfield 2014). However, some field botanists in Idaho question whether varietal distinction is justified by noting the existence of intermediate populations. These varieties may be similar enough as to represent two extreme forms on a continuum rather than distinct varietal forms. If they are distinct varieties, then they should have either distinct geographic distributions or distinct ecological settings or both. The purpose of this study is to investigate the morphological variation in relation to the geographical distribution and cationic composition of the soil substrate. More specifically, we test the hypothesis that the two morphologically recognized varieties, *calcareum* and *sceptrum*, are differentiated morphologically, geographically, and by their soil cation compositions, and should, thus, be recognized at the variety level.

METHODS

Morphological Characteristics

We measured four distinguishing characteristics (Reveal and Mansfield 2014)—scape length, leaf width, peduncle length, and vestiture coverage on

the involucre (as defined below)—in 27 accessions of *Eriogonum calcareum* from 25 outcrops across its full geographic range represented by specimens present in the College of Idaho’s Harold M. Tucker Herbarium (CIC, Table 2). Twenty-three unique outcrops were sampled, and two additional outcrops were sampled in two different years to check for inter-annual variation. Thus, accessions 11135 and 23963 are from the same outcrop in Elmore Co., and accessions 1468 and 14078 are from the same outcrop in Malheur Co. (Table 2). The longest scape, widest leaf, and longest peduncle were measured in each accession. Vestiture coverage on the involucre was measured as the ratio of the length of the involucre covered by floccose hairs to the total length of the involucre averaged for five involucres in each of two inflorescences per accession. Figure 1 illustrates these characters. Vestiture coverage data were arcsine square-root transformed before conducting statistical tests (Sokal and Rohlf 1995). Results were analyzed to determine statistical significance using the statistical software SigmaPlot 13.0 (Systat Software, Inc. San Jose, CA). Student’s t-tests were used except when data were not normally distributed. In those cases, a Mann Whitney U-test was performed. Populations having all characteristics unambiguously indicating either var. *calcareum* or var. *sceptrum* are hereafter referred

TABLE 2. Specimens of *E. calcareum* used in this study. Specimens are located in the Harold M. Tucker Herbarium (CIC). Involucre vestiture coverage is defined in the Methods section. Locations and geological formation age are estimated by geo-referencing data on herbarium label. Outcrops sampled for soils are indicated.

Accession	County	Latitude	Longitude	Variety determination	Soil collection	Scape length (cm)	Leaf width (cm)	Peduncle length (mm)	Involucre vestiture coverage	Geological epoch of deposit
2988	Owyhee	42.7224	115.7959	var. <i>sceptrum</i>		40.3	0.9	1.2	0	Pliocene
24795	Owyhee	43.308	116.5801	var. <i>sceptrum</i>		25	0.7	0.5	0	Holocene
342	Owyhee	42.8006	116.0229	var. <i>sceptrum</i>		26	0.8	0.63	0	Pliocene
12642	Owyhee	42.743	115.782	var. <i>sceptrum</i>		25.5	0.7	1	0	Pliocene
17273	Owyhee	43.0609	116.4611	var. <i>sceptrum</i>	H	36.8	1.1	1	0	Pleistocene
17493	Twin Falls	42.7459	114.9175	var. <i>sceptrum</i>	CN	28	0.8	0.6	0	Early Pleistocene and Late Pliocene
1843	Twin Falls	42.7022	114.8394	var. <i>sceptrum</i>		21.5	0.5	0.8	0	Pliocene
11135	Elmore	43.0028	115.2111	var. <i>sceptrum</i>		38.5	1	1.7	0.3	Late Pleistocene
7151	Elmore	43.0028	115.2111	var. <i>sceptrum</i>		30	0.5	1.5	0.2	Late Pleistocene
23963	Elmore	43.0028	115.2111	var. <i>sceptrum</i>		26.5	0.7	1.5	0.4	Late Pleistocene
19412	Owyhee	42.8006	115.7873	var. <i>sceptrum</i>	HC	26.3	0.7	2	0	Middle Pleistocene
33786	Owyhee	42.981	116.316	var. <i>sceptrum</i>	CC	34.8	0.9	1	0	Early Pleistocene and Late Pliocene
26710	Malheur	44.1775	117.2563	var. <i>calcareum</i>	MH	16.5	0.8	0.2	0.6	Miocene
43115	Payette	44.08	116.597	var. <i>calcareum</i>	BB	16.5	0.5	0.2	0.6	Pliocene
4765	Washington	44.16	116.63	var. <i>calcareum</i>		9	0.4	0.2	0.4	Pliocene
41577	Baker	44.59	117.4	var. <i>calcareum</i>	MC	15	0.5	0	0.4	Miocene to Pliocene
41587	Baker	44.51	117.41	var. <i>calcareum</i>	MC	8	0.4	0	0.6	Miocene to Pliocene
28266	Payette	44.0742	116.5446	var. <i>calcareum</i>		15	0.6	0.2	0.7	Miocene
4769	Baker	44.5851	117.5047	var. <i>calcareum</i>		9.3	0.8	0	0.7	Miocene to Pliocene
4766	Malheur	44.2352	117.3782	var. <i>calcareum</i>		23.5	0.5	0.2	0.8	Miocene to Pliocene
14078	Malheur	43.9327	117.6587	var. <i>calcareum</i>	HW	18.5	0.5	0.1	0.7	Miocene to Pliocene
32002	Malheur	43.927	117.66	var. <i>calcareum</i> approaching var. <i>sceptrum</i>		21.5	0.7	0.1	0.5	Holocene
4768	Malheur	43.9327	117.6587	var. <i>calcareum</i> approaching var. <i>sceptrum</i>	HW	17	0.5	0.3	0.3	Miocene to Pliocene
4770	Malheur	43.9034	117.2969	var. <i>calcareum</i> approaching var. <i>sceptrum</i>		27.5	0.6	0.7	0.5	Miocene to Pliocene
12641	Twin Falls	42.6569	114.8039	var. <i>sceptrum</i> approaching var. <i>calcareum</i>		22.5	0.9	0.5	0.2	Miocene
40528	Twin Falls	42.9081	114.9839	var. <i>sceptrum</i> approaching var. <i>calcareum</i>		27	0.8	2.2	0.1	Early Pleistocene and Late Pliocene
17395	Elmore	43.0173	115.2508	var. <i>sceptrum</i> approaching var. <i>calcareum</i>	OH	18	0.5	2.2	0.6	Early Pleistocene and Late Pliocene

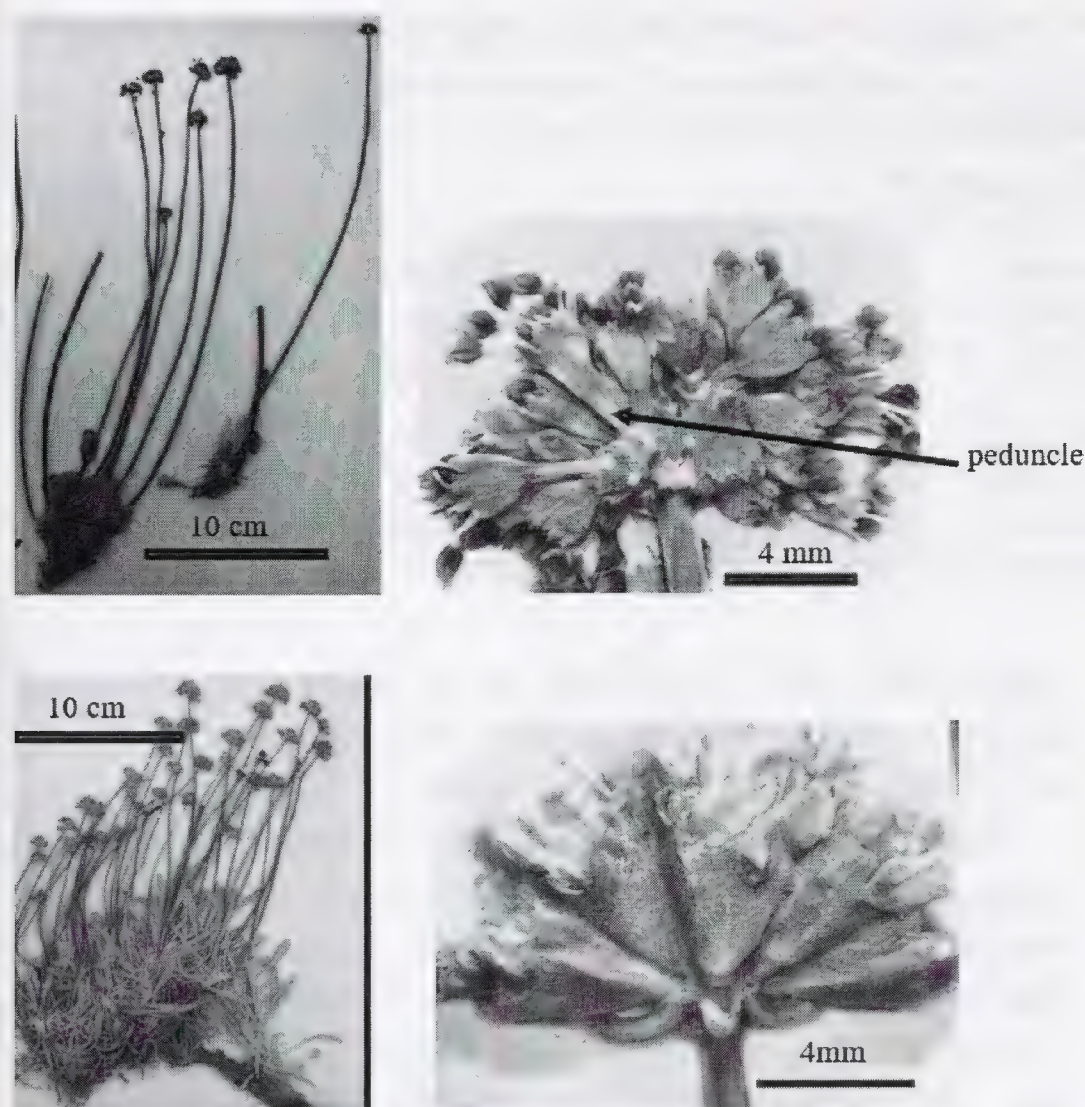


FIG. 1. Habits (left) and inflorescences (right) of 'consistent' var. *sceptrum* (top row) and 'consistent' var. *calcareum* (bottom row). Involucres of var. *sceptrum* are typically glabrous (vestiture coverage = 0 in the specimen illustrated) and on distinct peduncles; involucres of var. *calcareum* are typically quite floccose (vestiture coverage = 0.6 in the specimen illustrated) and sessile.

to as 'consistent' var. *calcareum* or 'consistent' var. *sceptrum*, respectively. In some cases, populations of otherwise 'consistent' var. *calcareum* possessed one of the characteristics typical of var. *sceptrum*. These populations are referred to as "var. *calcareum* approaching var. *sceptrum*." Similarly, some populations with all but one characteristic typifying var. *sceptrum* are referred to as "var. *sceptrum* approaching var. *calcareum*".

Study Sites

Soil samples were collected at nine outcrops occupied by populations, which were selected to represent the full range of geographical and morphological variation (Table 2). Three of these outcrops—Manning Creek (MC), Moores Hollow (MH), and Bannister Basin (BB)—were selected because they possess populations of 'consistent' var. *calcareum*. Four sites—Crow's Nest Road (CN), Hwy 78 (H), Hot Creek Road (HC), and Castle Creek (CC)—were selected because they possess populations of 'consistent' var. *sceptrum*. Finally, two sites—Harper-Westfall (HW) and Old Hwy 30 (OH)—were chosen as areas occupied by morphologically ambiguous populations of either var. *calcareum* approaching var. *sceptrum* or var. *sceptrum* approaching var. *calcareum*. Locations are identified in Table 2.

Soil Sampling

Three soil samples were collected at each of the nine soil study sites during either the summer of 2012 or winter of 2015. At each site, samples were collected randomly from within an area of 900 m² (depending on outcrop size) centered in the vicinity of the highest density of *E. calcareum* plants. After collection, all samples were placed in a drying oven for approximately 24 hr at 105°C, and then ground with a mortar and pestle to break apart large clumps.

Soil Composition Analysis

Three-hundred fifty grams from each soil sample were sent to the University of Idaho's Analytical Sciences Laboratory in Parma in either October 2012 or March 2015 for analysis of soil compositional properties. Properties analyzed were pH and the extractable cations: Mg, K, Ca, and Na. The mean and standard deviation of soil properties for each study site were calculated using the statistical computing program SigmaPlot 13.0 (Systat Software, Inc. San Jose, CA). Data from 'consistent' var. *calcareum* sites were then compared to data from 'consistent' var. *sceptrum* sites to evaluate statistical significance. These data were then compared again with the inclusion of the soils from the two ambiguous populations (HW and OH).

RESULTS

Morphological Characteristics

Twenty-one of the 27 populations of *E. calcareum* were unambiguously categorized as either var. *calcareum* or var. *sceptrum* (Table 2). In six populations at least one character was ambiguous. Populations were thus placed into the groups: 'consistent' var. *calcareum* (n = 9), 'consistent' var. *sceptrum* (n = 12), var. *calcareum* approaching var. *sceptrum* (n = 3), and var. *sceptrum* approaching var. *calcareum* (n = 3). Means of all four measured characteristics differed significantly between the two 'consistent' varieties (Table 3). When data were reanalyzed after including the six ambiguous specimens, the means changed slightly, however the levels of statistical significance of difference did not change (Table 3).

Geographical Distribution

Sites containing 'consistent' var. *calcareum* were all located in the northwestern portion of the *E. calcareum* range, all north the Snake River (or west as the river turns north near the Oregon-Idaho border, Fig. 2). Alternatively, sites containing 'consistent' var. *sceptrum* were all in the southeastern range of *E. calcareum* and all south of the Snake River (or north of it within half a mile in Elmore County). Outcrops throughout much of the southern extent of the range tended to be smaller and

TABLE 3. Morphological characteristics of the two varieties of *E. calcareum*. The first two columns include only ‘consistent’ specimens; the third and fourth columns (all) include the addition of morphologically ambiguous specimens. Values are means (one standard deviation); significance of first three rows was assessed by Student t-tests; last row by Mann Whitney U-test. * “Involucre vestiture coverage” is defined in the Methods section.

Character/taxon	Consistent var. <i>sceptrum</i>	Consistent var. <i>calcareum</i>	All var. <i>sceptrum</i>	All var. <i>calcareum</i>	Significance
Scape length (cm)	29.92 (6.10)	14.58 (5.07)	28.47 (6.46)	16.44 (5.91)	P < 0.001
Leaf width (cm)	0.76 (0.19)	0.54 (0.16)	0.77 (0.18)	0.57 (0.14)	P = 0.006
Peduncle length (mm)	1.11 (0.48)	0.12 (0.10)	1.22 (0.60)	0.18 (0.19)	P < 0.001
Involucre vestiture coverage*	0.08 (0.14)	0.61 (0.14)	0.12 (0.19)	0.57 (0.15)	P < 0.001

occasionally did not support any populations of *E. calcareum*. Sites occupied by morphologically ambiguous populations were located at the edge of the range of the variety. That is, sites occupied by var. *calcareum* approaching var. *sceptrum* are at the southwestern edge of the var. *calcareum* range, and sites occupied by var. *sceptrum* approaching var. *calcareum* are at the northeastern edge of the var. *sceptrum* range (Fig. 2).

Soil Analyses

Outcrops occupied by ‘consistent’ var. *calcareum* had significantly higher levels of extractable K (mean = 2.06 cmol/kg; P = 0.018) and Mg (mean = 10.50 cmol/kg; P < 0.001) than outcrops occupied by ‘consistent’ var. *sceptrum* (K mean = 0.74 cmol/kg; Mg mean = 3.03 cmol/kg; Table 4). When analyses included sites occupied by all populations—that is, those with ‘consistent’ and ambiguous morphologies—both K and Mg concentrations remained significantly different (P = 0.030 and P < 0.001, respectively). Furthermore, when soils from all populations were analyzed, those occupied by var. *calcareum* (including those approaching var. *sceptrum*) had significantly lower Ca/Mg (mean = 3.03; P = 0.016) and lower pH (mean = 5.15; P = 0.002) than soils occupied by var. *sceptrum* (including those approaching var. *calcareum*, Ca/Mg mean = 29.64; pH mean = 7.82) (Table 4).

DISCUSSION

The two varieties of *E. calcareum* can be distinguished morphologically (Table 3) using the characters identified by Reveal (1989) and Reveal and Mansfield (2014). The involucres of unambiguous (‘consistent’) *E. calcareum* var. *sceptrum* are nearly glabrous with floccose vestiture covering only 8% of the distal portion of the tube, and they are on peduncles that are 1.11 ± 0.48 mm long. Scares are (18-) 24–36 (-40) cm long; leaves are (0.5) 0.6–1.0 (1.1) cm wide. The involucres of unambiguous (‘consistent’) *E. calcareum* var. *calcareum* have floccose vestiture covering 61% of the distal portion of the involucre tubes, and the peduncles are nearly sessile (0.12 ± 0.10 mm long). Scares are (8-) 10–20 (-24) cm long; leaves are 0.4–0.8 cm wide. The most obvious difference in the field is that mature *E.*

calcareum var. *sceptrum* is a taller, more erect, narrower plant. Alternatively, var. *calcareum* appears as a more matted, broader plant (Fig. 1).

Populations with ambiguous character combinations had most morphological characteristics representative of one variety, but at least one of these characteristics was more typical of the other variety (Table 2). For example, var. *sceptrum* approaching var. *calcareum* specimens varied in the extent of vestiture on the involucres and peduncle length. That is, accession 17395 from Elmore Co. has the longer peduncle, broader leaves, taller scape, and more erect stature of var. *sceptrum*, but has the floccose distal involucre regions (vestiture coverage of 0.6) typical of var. *calcareum*. One accession from Twin Falls Co. (12641) has the short peduncle of var. *calcareum* but otherwise has the var. *sceptrum* phenotype. Two accessions from southern Malheur Co. (32002 and 2770) identified as var. *calcareum* approaching var. *sceptrum* had longer scape lengths (21.5–27.5 cm) than the ‘consistent’ var. *calcareum* specimens (8–23.5 cm).

Despite some ambiguity in morphological characters (Table 2), the two described varieties are completely allopatric, with var. *sceptrum* populations occurring further south and east in the range (Fig. 2). The outcrops occupied by the two varieties are comprised of sediments with differing origins (Table 2; Kittleman 1967; Swirydczuk 1977; Kimmel 1979; Izett 1981; Swirydczuk et al. 1982) and compositions (Table 4). Few studies have been conducted on the mineral compositions of these outcrops. Much of region occupied by var. *sceptrum* is in the Glenns Ferry Formation, which is comprised of lacustrine and fluvial sediments of Miocene to Pleistocene Lake Idaho (Idaho Group), including oolitic limestones (Swirydczuk 1977; Kimmel 1979; Swirydczuk et al. 1982). The region occupied by var. *calcareum* is similarly comprised of lacustrine and fluvial sediments of the Bully Creek (Kittleman 1967) and Deer Butte Formations (Corcoran et al. 1962), but being from the more western region of ancient “Lake Idaho”, apparently lacks the oolitic limestone deposits due to the depth and sedimentation patterns in the ancient lake. Although ‘consistent’ var. *calcareum* populations occupy sediments spanning earlier geological epochs (late Miocene to early Pleistocene) than ‘consistent’ var. *sceptrum* populations (Pliocene to mid-Pleistocene), the age of ashes is unlikely to be

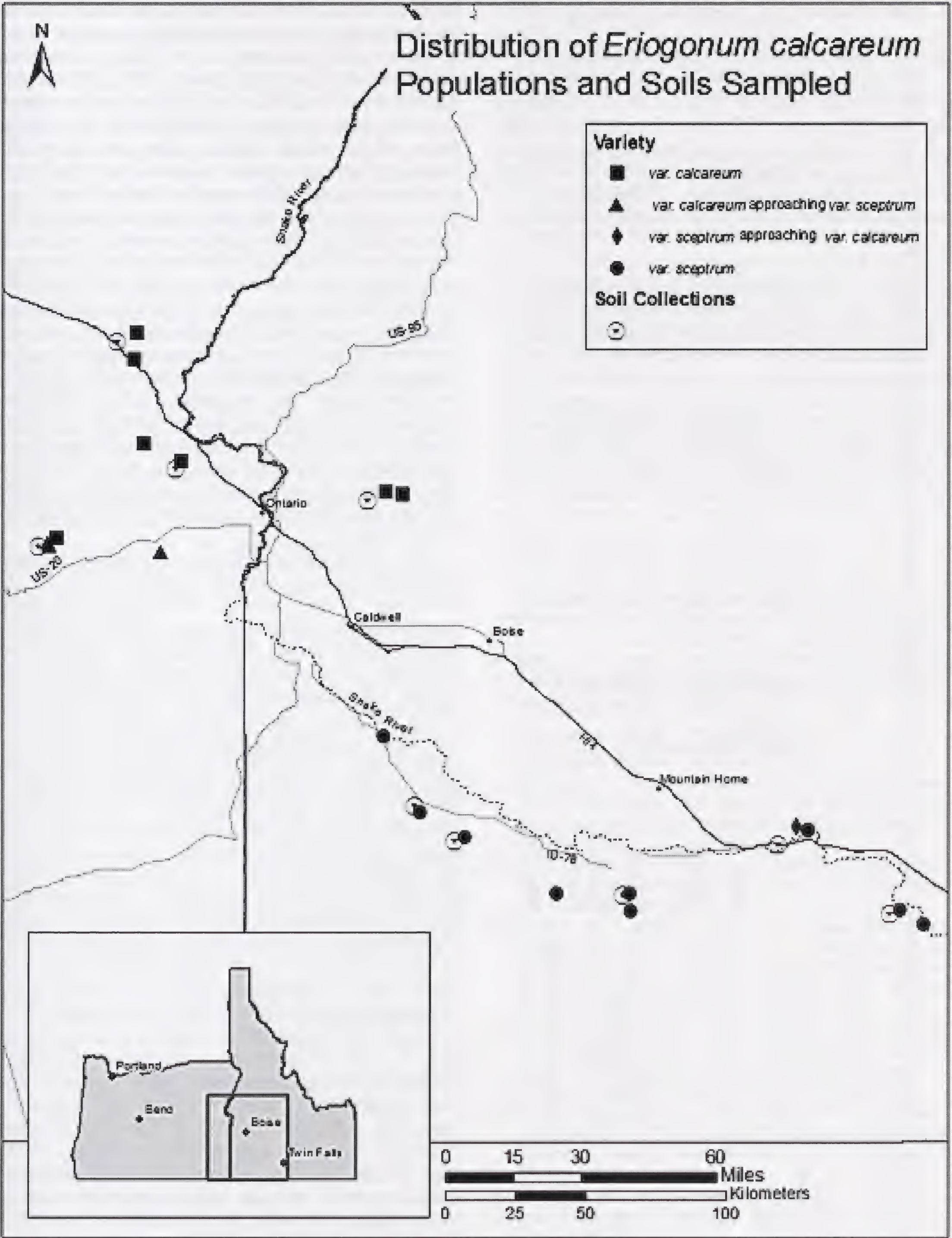


FIG. 2. Geographic locations of populations of the two varieties of *E. calcareum* and soil collection locations.

indicative of compositional differences. Populations of var. *sceptrum* occur on sites with both low Mg and low K concentrations that tend to have a slightly basic pH. Yet, the levels of Mg and K are not so low as to be in the range that normally limits plant growth (Epstein 1972). Populations of var. *calcareum* occur on sites with much higher acidity (more than 100 fold greater proton concentrations). Although

TABLE 4. Soil chemistry of the *E. calcareum* study sites. Values are means (one standard deviation); significance assessed by t-test: differences at $P < 0.05$ are denoted as “*”. All extractable cations measured as cmol/kg.

	Geological epoch	Ca	K	Mg	Na	pH	Ca/Mg
Northern (var. <i>calcareum</i>)							
Manning Creek Rd (MC)	Miocene to Pliocene	20.67 (4.93)	1.33 (0.25)	9.97 (1.84)	2.73 (0.40)	6.77 (1.02)	2.18 (0.83)
Moore's Hollow (MH)	Miocene to Pliocene	15.67 (4.04)	2.20 (0.35)	10.27 (4.73)	4.73 (3.00)	3.87 (0.21)	1.69 (0.52)
Bannister Basin (BB)	Pliocene	47.50 (31.44)	2.65 (0.84)	11.27 (2.74)	2.88 (1.35)	5.35 (0.30)	3.88 (1.86)
	Mean	27.94 (17.12)	2.06 (0.67)*	10.50 (0.68)*	3.45 (1.11)	5.35 (3.87)	2.58 (1.15)
Harper/Westfall (HW)	Miocene to Pliocene	34.33 (13.65)	5.17 (2.29)	7.6 (6.88)	3.10 (2.79)	4.60 (0.35)	9.85 (11.26)
	Mean All	29.54 (14.34)	2.84 (1.65)*	9.78 (1.55)*	3.36 (0.93)	5.15 (1.24)*	3.03 (1.81)*
Southern (var. <i>sceptrum</i>)							
Hwy 78 (H)	Pliocene	21.40 (15.40)	0.68 (0.59)	3.64 (3.68)	1.74 (2.83)	7.27 (0.06)	10.41 (7.83)
Castle Creek (CC)	Early Pleistocene and Late Pliocene	130.83 (100.25)	1.18 (0.08)	2.97 (0.79)	4.13 (5.36)	8.02 (0.38)	46.82 (35.91)
Hot Creek Rd (HC)	Middle Pleistocene	87.33 (58.56)	0.77 (0.04)	2.83 (0.45)	0.57 (0.08)	7.90 (0.00)	32.12 (21.64)
Crow's Nest Rd (CN)	Early Pleistocene and Late Pliocene	26.00 (0.00)	0.33 (0.08)	2.67 (0.59)	0.09 (0.00)	8.00 (0.20)	10.12 (2.51)
	Mean	66.39 (52.43)	0.74 (0.35)*	3.03 (0.43)*	1.63 (1.80)	7.95 (7.43)	24.87 (17.90)
Old Hwy 30 (OH)	Quaternary (Pleistocene)	47.33 (25.70)	1.13 (0.12)	1.93 (0.65)	0.09 (0.00)	7.90 (0.10)	29.64 (25.75)
	Mean All	62.58 (46.20)	0.82 (0.35)*	2.81 (0.61)*	1.32 (1.71)	7.82 (0.13)*	29.64 (10.27)*

the var. *calcareum*-occupied outcrops have significantly higher levels of Mg and K, the levels are below those normally considered to be toxic to most plant functions (Epstein 1972; Brooks 1987). The higher Ca/Mg levels and higher pH of the var. *sceptrum*-occupied outcrops may be explained by the intercalation of the silicic volcanic ashes throughout the oolitic calcareous deposits characterizing the more southeastern part of the western Snake River Plain. Indeed, some of the var. *sceptrum*-occupied outcrops (HC and CC in Table 3, most notably) had much higher levels of Ca than other sites tested. Although Izett (1981) has demonstrated that the ash falls throughout this region are of varying origins and compositions, no detailed mapping of the outcrops occupied by any of the *E. calcareum* populations has been performed. Regardless of whether the variation in substrates of the two varieties of *E. calcareum* originated from differing source materials or subsequent processes that these geologic units may have undergone, or both, the important point is that the morphologically and geographically distinguishable varieties occupy soils of significantly different chemistry (Table 4).

The biological significance of these chemical differences among the outcrops occupied by *E. calcareum* is not clear. It is possible that some morphological differences may result from non-genetic effects rather than adaptation. Higher Ca/Mg ratios are known to increase plant growth (stem height) experimentally in willows (Mleczek et al. 2012). The longer scapes of var. *sceptrum* (than var. *calcareum*) may result from a direct phenotypic effect of the higher Ca/Mg ratio in outcrops they occupy. Alternatively, at least some morphological differences may result from adaptation resulting from different selection pressures experienced by populations of the two different varieties. Numerous studies investigating endemism on challenging substrates, including serpentine (Jenny 1980; Kruckeberg 1985; Brooks 1987; Kruckeberg 2002; Taylor et al. 2002; Harrison and Rajakaruna 2011), gypsum (Moore et al. 2014; Escudaro et al. 2015), shales (Silva and Ayers 2016), and gabbro (Medeiros 2015) have demonstrated that extreme mineral compositions or physical soil properties can impose selection pressures on plant populations and drive significant adaptation (Rajakaruna et al. 2003; Sambatti and Rice 2006, 2007), which, in turn can stimulate speciation (Harrison et al. 2004; Brady et al. 2005; Anacker et al. 2011; Kay et al. 2011). It is possible that varietyally distinct populations of *E. calcareum* studied here are likewise being subjected to significantly different edaphic environments to the extent that adaptation can occur. The levels of minerals in the outcrops occupied by *E. calcareum* are unlike those in many environments previously studied where adaptation has been demonstrated. For example, the lowest Ca/Mg ratio observed in this study (1.7; Table 4) is still within the range that normally imposes no stress to plant functions (values

> 1; Epstein 1972; Brooks 1987) and well above those levels considered chemically challenging in ultramafic soils (0.04–0.2; Brooks 1987). Levels of Ca, Mg, Na, and K in all outcrops examined in this study, while variable, are not outside of the range considered normal for plant growth (Epstein 1972). There still may be selection forces that operate differently, albeit more slowly than in ultramafic sites, in the outcrops occupied by the two varieties of *E. calcareum*. There are many components of soil chemistry and physics of these outcrops that have not been studied yet.

While differential edaphic selection may be low between the varieties, the barren nature of the ash outcrops throughout southwestern Idaho and southeastern Oregon, including those occupied by *E. calcareum*, may increase the likelihood of selection. Sambatti and Rice (2006) have demonstrated in ultramafic sites that local adaptation is only evident in the absence of competition, so the low cover of vascular plants evident in these ash outcrops may contribute to more rapid selection. And although the particular stressors to vascular plants in the ash beds of this region have not been ascertained (Grimes 1987), the edaphic environments are unproductive and, as such, minimize competition (Harrison and Rajakaruna 2011).

Some of the morphological differentiation seen in *E. calcareum* (Table 3) may result from isolation of the varieties from one another. Insofar as isolation reduces gene flow (Futuyma 1997), the patchy, isolated nature of the barren outcrops occupied by *E. calcareum* can enhance genetic differentiation of varieties. Distances between the varieties on either side of the western Snake River Plain are greater than those from one outcrop to another within the range of each variety. That is, the outcrops in the northwestern end of the range (those occupied by var. *calcareum*) are no closer than 84 km from the closest outcrops in the southeastern part of the range (those occupied by var. *sceptrum*—Fig. 2). Distances between adjacent *E. calcareum* var. *sceptrum*-occupied outcrops vary from approximately 1 km to no more than 50 km. And distances between adjacent *E. calcareum* var. *calcareum*-occupied outcrops range from a few hundred m to no more than 50 km. Though there may be populations of *E. calcareum* on unexplored outcrops in either the southeastern (var. *sceptrum* range) or northwestern (var. *calcareum* range) regions of the species' range, there are no outcrops in the Quaternary sediments of the now heavily developed region of the Treasure Valley between these two sets of populations. Thus, the spatial distribution of substrates likely contributes to the differentiation of varieties in *E. calcareum*, regardless of the strength of selection. However, the importance of geographic separation and its effect on gene flow relative to selection and adaptation or other factors that may contribute to varietal differentiation cannot be adequately assessed here without

evaluating genetic interchange or pollinator interaction.

The genus *Eriogonum* is one of the most diverse plant genera in western North America, and it is considered to be rapidly evolving (Reveal 2005). Many of the approximately 250 species of *Eriogonum* are narrowly restricted to specific substrates (Reveal 2005). In *E. calcareum* the geographic separation of patchy outcrops of chemically distinct substrates contributes to evident morphological differentiation of varieties.

For recently evolving populations, such as *Eriogonum calcareum* var. *calcareum* and var. *sceptrum*, geographic separation and variations in soil chemistry are likely modifying the rate of natural selection and morphological differentiation within the species. These two varieties offer an example of the ecological and geographic differentiation between plant populations of a rapidly evolving genus in North America. This study also demonstrates the potential of the archipelago of edaphic islands in southwestern Idaho and southeastern Oregon as a laboratory for the study of vascular plant evolution.

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ASSESSING WATER USE VARIATION AMONG THE CYTOTYPES OF THE
AUTOPOLYPLOID SOUTHWESTERN DESERT CREOSOTEBUSH (*LARREA*
TRIDENTATA [DC.] COVILLE: ZYGOPHYLLACEAE)

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ABSTRACT

Genome duplication, or polyploidy, has played an important role in the diversification of flowering plants, but the ecological and evolutionary consequences of polyploidy still remain unclear. Polyploidy is known to either cause or facilitate phenotypic changes, and ploidy-specific phenotypic differences may lead to the exploitation of novel niche space. Many studies reporting phenotypic variation between cytotypes have been observational, and may not reflect ecological adaptation by diploids and polyploids to different habitat. One such trait, water use, may play an outsized role in survival and population expansion into novel habitat. To test whether water use differs among ploidy levels, we grew field-collected diploid, autotetraploid, and autohexaploid cytotypes of the characteristic North American desert plant, *Larrea tridentata* (DC.) Coville, under greenhouse conditions. We measured whole plant water use gravimetrically over six wk, standardizing water use with measures of total stomatal area. We found that water use was positively correlated with stomatal area, but the cytotypes had similar total stomatal areas and did not differ significantly in mean water use/total stomatal area. Cytotype-specific water use responses through time were also not significantly different. Taken together, these results suggest that the cytotypes have similar water relationships, and possibly fitness outcomes, with respect to water use in common environments.

Key Words: desert plant, leaf area, polyploidy, stomatal area, stomatal density, water use.

Paleontological and genomic studies indicate the evolutionary history of angiosperms is punctuated by repeated polyploidization, or whole genome duplication events (Masterson 1994; Ramsey and Schemske 1998; Soltis et al. 2009). Contemporary opinion implicates genome duplication as having played an important role in the ancient diversification of flowering plants (*Amborella* Genome Project 2013). As an evolutionary force, polyploidy continues to play a role in plant diversification with approximately 15% of speciation events involving genome duplication (Wood et al. 2009). Genome duplication may also cause phenotypic alterations between polyploids and their diploid progenitors. Differences in herbivore resistance, phenology, reproductive system, cell size, and drought tolerance have been documented between diploids and polyploids (Bretagnolle and Lumaret 1995; Segraves and Thompson 1999; Maherali et al. 2009; Ramsey 2011). Arising either as a direct effect of increased chromosomal complement (Levin 1983, 2004), or from adaptative responses to extrinsic factors (Maherali et al. 2009; Ramsey 2011; Madlung 2013), such differences have been argued to facilitate polyploid expansion into novel habitat by broadening ecological amplitude (Brochmann et al. 2004; Martin and Husband 2009; McIntyre 2012; but see Buggs and Pannell 2007) and

may lead to ecological speciation (Levin 2004; Ramsey 2011; Martin and Husband 2013).

Though polyploidy may recurrently introduce genetic and phenotypic variation into populations, the ecological consequences of genome duplication for adaptation remain unclear (Ramsey and Ramsey 2014; Soltis et al. 2014). Many studies reporting phenotypic variation between cytotypes have been observational, focused on climatic correlates with diploid and polyploid distributions, for example, or on simple trait differences between cytotypes (Ramsey and Ramsey 2014; Soltis et al. 2016). Ecological niche modeling studies of diploid and autotetraploid *Chamerion angustifolium* (L.) Holub indicate tetraploids are found in warmer and drier habitats than diploids, consistent with ecological adaptation associated with polyploidization (Thompson et al. 2014). Recent experimental efforts building upon observational studies have made great strides in characterizing the fitness consequences of apparent ecological adaptation by polyploids. For example, water relations, particularly important for plant adaptation and ecological niche differentiation (Heschel et al. 2002; McKay et al. 2003; McKay et al. 2008; Engelbrecht et al. 2007), have been investigated in a number of systems comprising multiple cytotypes. Allotetraploid *Brachypodium hybridum* Catalán, Joch. Müll., Hasterok & G. Jenkins is more often found in arid habitats than diploid *Brachypodium distachyon* (L.) P. Beauv., and greenhouse studies show that the tetraploids have greater water use efficiency than diploids (Manzaneda et al. 2012;

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Manzaneda et al. 2015). Similarly, reciprocal transplant experiments with tetraploid and hexaploid *Achillea millefolium* L. demonstrate ecological adaptation by the two cytotypes (Ramsey 2011), and common garden experiments document differences in altitudinal adaptation (Martin and Husband 2013) and water relations (Maherali et al. 2009) between diploid and tetraploid *C. angustifolium*. However, such relationships are not universal. Reciprocal transplant studies of *Allium oleraceum* L. indicate less clear ecological differentiation among cytotypes (Duchoslav et al. 2016). Rather, individuals tended to show some evidence of local adaptation, regardless of ploidy, and that tetraploids, pentaploids, and hexaploids may exhibit enough phenotypic plasticity to cope with the ecological variation present throughout the entire range of the species. Thus, what seems clear from the emerging literature exploring diploid and polyploid ecological differences is the need for additional experimental evaluations of ecologically relevant phenotypic differences.

Phenotypic alterations associated with genome duplication that alter water relations may play an outsized role in facilitating range expansion, ecological adaptation, and community assembly (Cavender-Bares et al. 2004; Levin 2004; Ramsey 2011; McIntyre 2012). Numerous taxa comprising intra-specific ploidy levels are known to have distributions correlated with climatic or other ecological gradients (e.g., *Ambrosia* L., Raven et al. 1968; *Larrea* Cav., Yang and Lowe 1968; *Melampodium* L., Stuessy 1971; *Tolmiea* Torr. & A. Gray, Soltis 1984; *Chamerion* [Raf.] Raf. ex Holub, Husband & Schemske 1998; *Galax* Sims, Burton and Husband 1999; *Achillea* L., Ramsey et al. 2008; *Campanula* L., Sutherland and Galloway 2016). However, in most cases inter-cytotype phenotypic differences that could facilitate niche partitioning along ecological gradients, including water use, remain poorly known and often have not been examined in common environments. Traits such as water use efficiency (water use/carbon gain) can play an important role in determining species distributions by influencing carbon assimilation and fitness (Hetherington and Woodward 2003; McKay et al. 2008; Hodgson et al. 2010; Kropp and Ogle 2015). However, measurements of water use efficiency typically involve sensitive equipment, may be influenced by slight methodological variations, and are often difficult to extrapolate to whole-plant measures of water use efficiency under natural conditions for large perennial plants that may exhibit interleaf variability in transpiration and photosynthetic rates, or when age and ecological history is unknown (Percy et al. 1989; Cirelli et al. 2012). In contrast, estimates of whole plant water use using gravimetric methods can provide a snapshot of plant water relations under a range of conditions despite lacking an estimate of carbon assimilation (Cirelli et al. 2012; Medrano et al. 2015). Here, we investigated water use differences between the cytotypes of one archetypal autopoly-

ploid desert plant in an effort to more clearly understand the role genome duplication has played in ecological differentiation.

Larrea tridentata (DC.) Coville (Zygophyllaceae) is a widespread and ecologically dominant species of the North American warm deserts (Hunziker et al. 1977; Lewis 1980). The ecological success of *L. tridentata* in arid environments has been attributed to its ability to maintain photosynthesis during extreme heat and drought (Ogle and Reynolds 2002). For example, studies in the Chihuahuan Desert indicate *L. tridentata* can sustain pre-dawn water potentials (Ψ_{pd}) in excess of -10 Mpa (Cunningham and Burk 1973), while maintaining positive net photosynthesis (Odening et al. 1974). Similarly, plants in the Mojave Desert have been recorded maintaining positive net photosynthesis while experiencing water potentials as low as -60 atm (Bamberg et al. 1973). Not considered in these studies is that *L. tridentata* comprises three autopolyploid chromosome races distributed throughout the Chihuahuan (diploids; $2n = 2x = 26$), Sonoran (predominantly tetraploids $2n = 4x = 52$), and Mojave (predominantly hexaploids; $2n = 6x = 78$) Deserts of the southwestern U.S. and northern Mexico (Yang 1967, 1970; Yang and Lowe 1968; Barbour 1969; Hunter et al. 2001; Laport et al. 2012; Laport et al. 2013; Laport and Ramsey 2015). Though hypothesized to have facilitated adaptation to increasingly arid environments (Barbour 1969; Hunziker et al. 1977), the influence of genome duplication on the ecological success of *L. tridentata* remains essentially untested for many ecologically relevant traits (Laport et al. 2016).

Prior studies of *L. tridentata* indicate the cytotypes evolved relatively recently (ca. ≤ 1 mya; Laport et al. 2012), but occupy climatically distinct habitats even in areas where they come into contact (Barbour 1969; Yang 1970; Laport and Ramsey 2015). Differences in leaf, flower, and whole-plant traits among the cytotypes (Laport and Ramsey 2015) support hypotheses that repeated genome duplications have contributed to phenotypic differentiation (Barbour 1969), and facilitated rapid ecological adaptation (Yang 1970; Hunziker et al. 1977). However, these morphological differences do not always vary linearly with increasing ploidy, and may be uncoupled from physiological traits related to water use. For example, Barbour (1969) observed that diploid seedlings were more drought tolerant than tetraploid and hexaploid seedlings. Laport et al. (2013) found that tetraploids and hexaploids occupy climatic niches that are significantly different from their diploid and tetraploid progenitors, respectively, but also that the cytotypes tend to occupy climatically similar areas within their respective deserts. Combined, these prior observations leave unclear the degree to which genome duplication has altered cytotype-specific water use and facilitated adaptation to the unique ecological conditions of the Chihuahuan, Sonoran, and Mojave Deserts. To investigate whether water use differs among cytotypes of *L.*

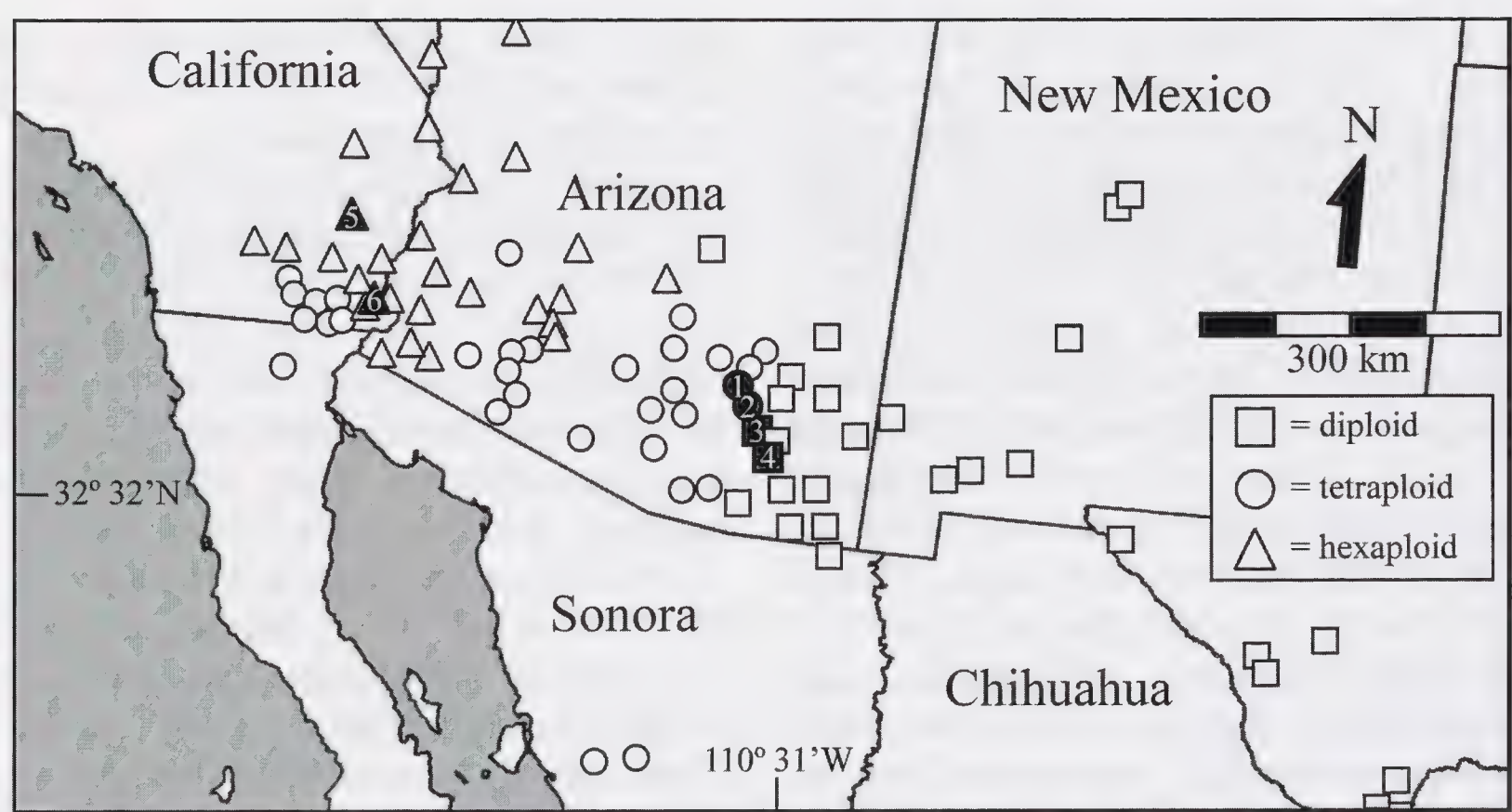


FIG. 1. Map of live *L. tridentata* collection localities. The distributions of diploid (open square), tetraploid (open circle), and hexaploid (open triangle) *L. tridentata* throughout the southwestern US and northern Mexico was previously determined by Laport et al. (2012) and Laport and Ramsey (2015) using flow cytometry to infer the ploidy of >20 adult plants at permanent study sites. Filled site markers (numbered 1–6) indicate where seedlings were collected for water use analyses. Numbering of sites corresponds to Table 1.

tridentata, we grew field-collected plants of known ploidy under greenhouse conditions and measured whole plant water use gravimetrically. Making these investigations in *L. tridentata*, an ecologically dominant member of the North American desert flora, will help shed light on the evolutionary significance of polyploidy to ecological adaptation, incipient speciation, and patterns of biodiversity, as well as elucidate the structuring of desert plant communities.

MATERIALS AND METHODS

Live Plant Collections

In the spring of 2014, we collected small, naturally occurring diploid, tetraploid, and hexaploid plants (ca. 5–15 cm tall) from each of two previously identified sites of known ploidy (six sites total, Fig. 1, Table 1; Laport et al. 2012; Laport and Ramsey 2015). We were unable to make collections at additional sites because seedling recruitment and survival is episodic for *L. tridentata* (Chew and Chew 1965). Seedlings were transported to the University of Nebraska-Lincoln greenhouses in 7.5 cm peat pots

containing native soils. Of the plants that survived being transported we re-potted (along with some of the native soil) 15 diploids, 15 tetraploids, and 15 hexaploids into 13 cm plastic pots with a growing media consisting of five parts Sunshine MVP (formerly Metro-Mix 200, www.hummert.com), three parts washed horticultural river sand, and three parts calcined clay (Turface MVP, www.hummert.com). Plants were irrigated once a week and fertilized once per month for nine months under common greenhouse conditions prior to water use assessments.

Water Use and Transpiration Determination

To determine if water use differed among the three cytotypes, we measured whole plant water loss gravimetrically every week for six weeks. We assigned potted plants of each ploidy to shuttle trays (Fig. 2A). Each week, the potted plants were given 200 ml of water, ensuring no water leaked from the bottom of the pots. After watering, we determined the mass of each pot (“initial mass”), and then allowed the plants to transpire for 72 hr. We then

TABLE 1. Environmental conditions at collection localities for live *L. tridentata* plants. All environmental data from WorldClim (www.worldclim.org; Hijmans et al. 2005). Site names and cytotypes (in parentheses) correspond to permanent collection sites established by Laport et al. (2012) and Laport and Ramsey (2015).

Sites	Latitude (°N)	Longitude (°W)	Elevation (m)	Mean annual temperature (°C)	Mean annual precipitation (mm)
1. San Pedro 1 (4x)	32.633983	-110.55865	809	19.5	351
2. San Pedro 2 (4x)	32.616083	-110.5386	780	19.3	347
3. San Pedro 4 (2x)	32.538133	-110.50795	815	19.1	356
4. San Pedro 5 (2x)	32.555917	-110.516267	810	19.3	348
5. Joshua Tree (6x)	33.67395	-115.800983	481	20.4	122
6. Algodones S4 (6x)	32.8886	-114.842067	166	22.2	74

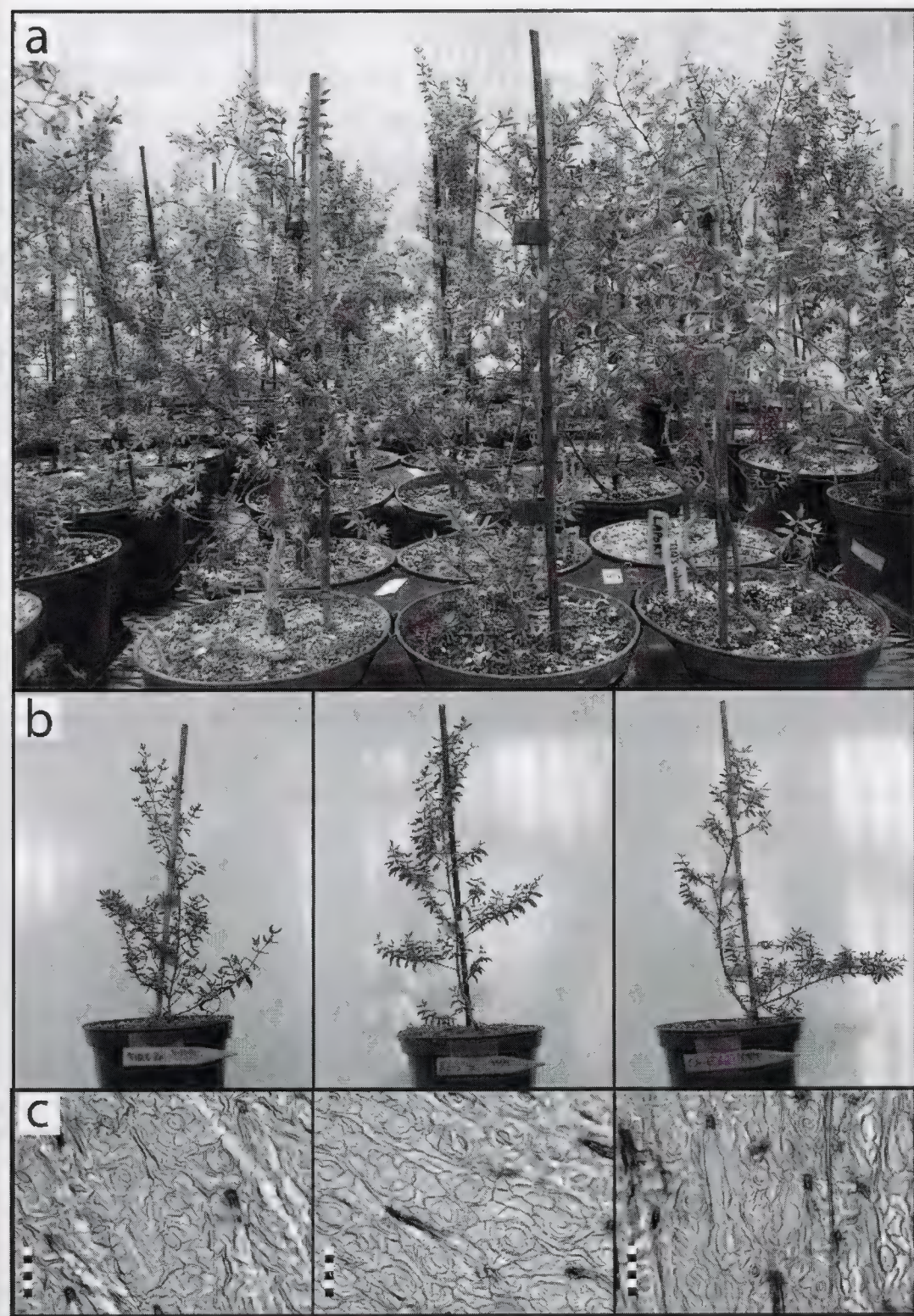


FIG. 2. Wild-collected *L. tridentata* in the experimental water use setup. A) Diploids, tetraploids, and hexaploids randomly arrayed in shuttle trays for assessment of whole plant water use. B) From left to right, diploid (2 \times), tetraploid (4 \times), and hexaploid (6 \times) plants, against a white background for leaf area assessments. Total leaf areas were approximately equal among cytotypes. C) From left to right, images of diploid, tetraploid, and hexaploid epidermal impressions used for stomatal area and density measures (400 \times total magnification). Stomatal size increased with ploidy, but stomatal density decreased with increased ploidy. Scale bars in each epidermal impression image measure $\sim 40\ \mu\text{m}$.

determined the mass of each of the potted plants again (“final mass”). After determining the final mass, the shuttle trays were shifted to reduce positional bias in lighting and airflow within the greenhouse between trials, as some trays did not contain all of the cytotypes. The plants were then watered lightly to provide sufficient water until the beginning of the next trial. We determined total whole plant water loss as the difference between the initial and final masses.

To estimate the whole plant water use, we corrected for water lost to evaporation using four control pots containing only the growing media. The control pots were distributed among the shuttle trays in a representative way, and were watered and massed in the same manner as the potted plants. The change in mass among the control pots was

averaged each week and subtracted from the weekly total water loss by each of the potted plants as evaporated water. We considered the remaining value as total water use by the potted plants.

Total Leaf Area Determination

Transpiration in *L. tridentata* is sensitive to leaf area (Ritchie 1972; Bird 2010). To reduce the influence of leaf area on water use across individuals, we lightly pruned the plants periodically to attain approximately similar overall sizes prior to water use assessments. We estimated the total leaf area for each of the potted plants during the third week of the experiment to capture the leaf area at the midpoint of any growth that may have occurred over the six-week experiment. To estimate the total leaf area, we photographed each plant against a white background along with a scale of known length ensuring visible leaf area appeared representative for a two dimensional image (Fig. 2B). Each image was then imported into ImageJ (Schneider 2012) and transformed into a binary image. We set the scale for each image using the length of the known scale in each image, and then determined the total leaf area of the plant image in mm^2 .

Stomatal Size and Density

Stomatal size and density are correlated with ploidy (Masterson 1994; Hunter et al. 2001; Beaulieu et al. 2008), and play an important role in water use efficiency (Hetherington and Woodward 2003). We made adaxial epidermal leaf impressions at the conclusion of the study (week six) by coating the surface of three haphazardly chosen pressed and dried leaves with a thin layer of clear nail polish. The nail polish was allowed to dry prior to being peeled off the leaf with clear plastic tape and affixed to a glass microscope slide for determination of stomatal area and density with a compound light stereomicroscope (Carl Zeiss Axio-star Plus) under 400 \times total magnification (Barbour et al. 1974; Hunter et al. 2001; Fig. 2C). Digital photographs for a representative field of view for each leaf were made using a digital camera affixed to the microscope (Leica EC3 paired with Leica Acquire software v3.2). We counted stomatal density and measured the area of three sets of stomatal guard cells for three leaf impressions for each of the 45 greenhouse-grown plants (405 total stomatal guard cell areas) using ImageJ (Schneider 2012). Area measures were converted to μm^2 using a calibrated stage micrometer viewed at the same magnification. All measurements were averaged for each plant and used to estimate total stomatal area for each plant by multiplying by the leaf area estimates. This allowed us to standardize measurements of water use across plants by determining the mean water use/total stomatal area ($\text{grams/hr}/\mu\text{m}^2$) for each plant.

Data Analysis

We evaluated the relationship between water use and total stomatal area, with a linear regression. We tested for cytotype differences in leaf area, stomatal area, and stomatal density with a MANOVA model that included ploidy as a categorical effect. Individual differences were evaluated with ANOVA and a Tukey HSD test for post hoc comparisons between cytotypes. To test whether the cytotypes differed in mean water use/total stomatal area (averaged across the six wk of the study) we used an ANOVA model that included ploidy as a categorical effect and a Tukey HSD test for post hoc comparisons between cytotypes. To examine changes in water use over time we implemented a repeated measures ANOVA model that included ploidy, date, and ploidy \times date as effects. Stomatal areas and densities, and mean water use/total stomatal area were natural log transformed prior to statistical analyses to improve the distribution of residuals. All statistical analyses were performed in JMP Pro (v.12; SAS Institute Inc., Cary, NC, USA).

RESULTS

The small plants in this experiment were collected near the distributional contacts between the three cytotypes (Fig. 1). Though generally abiotically similar, the diploid collection sites tended to be at higher elevations with slightly cooler mean annual temperatures and slightly higher mean annual precipitation than the tetraploid and hexaploid collection sites. The hexaploid collection sites occurred at the lowest elevations and had the highest mean annual temperatures and lowest mean annual precipitation (Table 1). The cytotypes had significantly different leaf and stomatal features (Wilks' $\lambda = 0.225$, $F_{6,80} = 14.772$, $P < 0.0001$). Although the cytotypes did not differ in total leaf area ($F_2 = 0.186$, $P = 0.831$), they were significantly differentiated by individual stomatal area ($F_2 = 65.156$, $P < 0.0001$) and density ($F_2 = 25.131$, $P < 0.0001$). Tetraploids had the greatest mean leaf area ($20,873.79 \text{ mm}^2$, $\text{SE} = 2320.57 \text{ mm}^2$), followed by diploids ($19,836.12 \text{ mm}^2$, $\text{SE} = 1356.22 \text{ mm}^2$) and hexaploids ($19,451.33 \text{ mm}^2$, $\text{SE} = 1237.14 \text{ mm}^2$). Diploids had the lowest individual stomatal area ($167.006 \text{ } \mu\text{m}^2/\text{stomata}$, $\text{SE} = 3.647$) and the highest stomatal density ($4.203 \times 10^{-4}/\mu\text{m}^2$, $\text{SE} = 1.347 \times 10^{-5}$), followed by tetraploids ($199.279 \text{ } \mu\text{m}^2/\text{stomata}$, $\text{SE} = 3.472$; $3.800 \times 10^{-4}/\mu\text{m}^2$, $\text{SE} = 1.271 \times 10^{-5}$), and hexaploids ($275.257 \text{ } \mu\text{m}^2/\text{stomata}$, $\text{SE} = 7.348$; $2.803 \times 10^{-4}/\mu\text{m}^2$, $\text{SE} = 1.406 \times 10^{-5}$). These measures differ from those reported previously by Barbour et al. (1974) and Hunter et al. (2001), which may be related to the genetic variability expressed at the specific localities we sampled, or to having grown the plants for an extended period in the greenhouse. Nevertheless, we observed the same relative size and density relationships among cytotypes. Mean water use was posi-

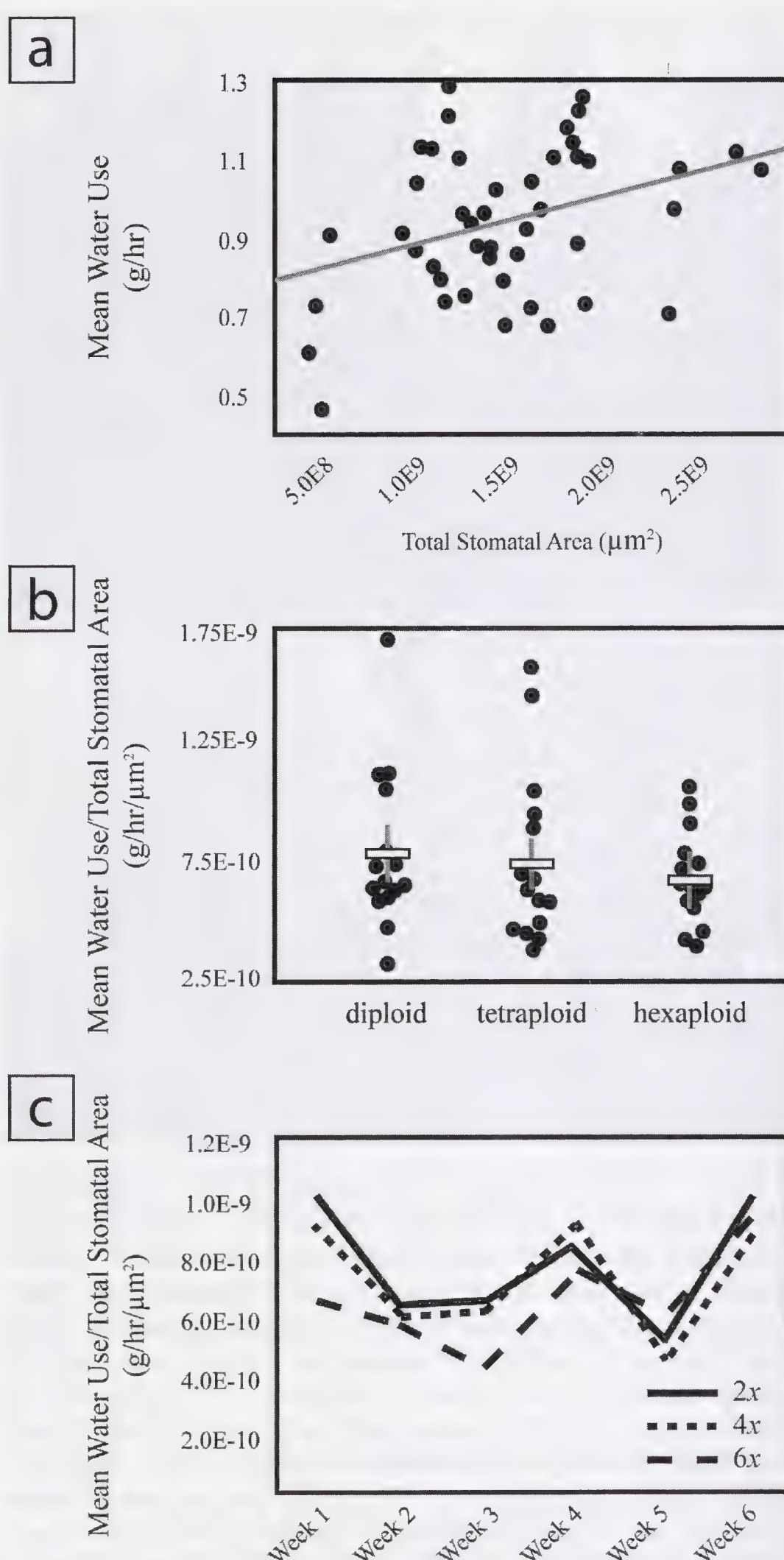


FIG. 3. Water use relationships for the three cytotypes of *L. tridentata*. A) Mean water use was positively correlated with total stomatal area. B) Diploids, tetraploids, and hexaploids did not differ in mean water use/total stomatal area. Each point indicates an individual plant mean over the six weeks of the experiment. Mean values are indicated with a rectangle, and error bars indicate ± 1 SE. C) Diploids, tetraploids, and hexaploids varied in mean water use/total stomatal area over the course of the experiment. However, cytotype-specific responses over time were not significantly different in a repeated measures analysis.

tively correlated with total stomatal area ($R^2 = 0.125$, $P = 0.017$), despite a large spread in water use values (Fig. 3A). However, while diploids had slightly higher mean water use/total stomatal area than tetraploids or hexaploids, the cytotypes did not differ significantly in mean water use/total stomatal area in our ANOVA model ($F_2 = 0.3975$, $P = 0.675$; Fig. 3B).

Over the six weeks of the experiment, the mean water use/total stomatal area varied differentially among the three cytotypes (Fig. 3C). While water use fluctuated approximately equally up and down from week one to six, diploids and tetraploids initially had slightly higher mean water use/total stomatal area than hexaploids, but by the end of the experiment this relationship disappeared. Despite these fluctuations, the repeated measures model indicated that none of the cytotypes significantly differed in mean water use/total stomatal area \times date interactions (Wilks' $\Lambda = 0.700$, $F_{10, 76} = 1.485$, $P = 0.161$). Thus, the cytotypes had similar water use responses over time (Fig. 3C).

DISCUSSION

Studies over the last few decades make clear that polyploidy is an important evolutionary mechanism in angiosperms (Soltis et al. 2009; Madlung 2013; Ramsey and Ramsey 2014; Husband et al. 2016). Since the discovery of polyploidy, evolutionary biologists have sought to determine whether observed trait differences between diploids and polyploids are an intrinsic consequence of genome duplication or result from ecological adaptation (Levin 1983; Ramsey and Ramsey 2014; Soltis et al. 2014). Though important for understanding the evolution of polyploid species, this question has proven difficult to parse. Extant polyploid species often comprise cytotypes that have already diverged over many generations, and the artificial creation of neopolyploids may not adequately represent the combination of alleles present at the formation of natural polyploids (Husband et al. 2016). Ecological differences may also exist among allopolyploid cytotypes that are not observed immediately upon formation among autopolyploid cytotypes since allopolyploids combine two diverged genomes (Ramsey and Schemske 1998). For example, allopolyploids may more often exhibit patterns of ecological niche differentiation at broad scales than autopolyploids (Glennon et al. 2014; Marchant et al. 2016). Nevertheless, comparisons of relatively recently diverged cytotypes in common garden experiments and reciprocal transplants have been instrumental in better understanding the role genome duplication plays in ecological divergence (Ramsey 2011; Martin and Husband 2013; Duchoslav et al. 2016; Segraves and Anneberg 2016).

The renewed focus on studying polyploid evolution has resulted in the recognition that intraspecific ploidy levels often represent novel genetic and phenotypic entities (Ramsey and Ramsey 2014). Recent studies have characterized cytotype-specific genetic (Vallejo-Marín and Lye 2013; Fehlberg and Ferguson 2012; Glennon and Church 2015; Laport et al. 2016), climatic (Glennon et al. 2014; Thompson et al. 2014), ecological (Ramsey 2011; Martin and Husband 2013), pollinator (Husband and Schemske 2000; Nghiem et al. 2011; Borges et al. 2012;

Roccaforte et al. 2015), and herbivore (Thompson and Merg 2008) differences, but such differences have been investigated for relatively few species in common environments, and the underlying mechanisms for observed differences remain understudied. As a measure of physiological performance, water use relations have proven useful for understanding cytotype divergence. In allopolyploid species, such as *Brachypodium* Brid., pronounced cytotype-specific differences in water use may have facilitated niche partitioning by diploids and tetraploids in the Mediterranean basin (Manzaneda et al. 2012; Manzaneda et al. 2015). However, differences in water use between diploid and autotetraploid *C. angustifolium* are less evident (Maherali et al. 2009), and may only have a limited effect on intercytotype competition even when water is limited (Thompson et al. 2015). Such observations suggest polyploid physiological adaptation may be complex, and that other ecological interactions may play a significant role in cytotype divergence resulting in idiosyncratic outcomes with respect to the ecological setting or whether auto- or allopolyploidy was involved.

Water use has been assessed previously in *L. tridentata* to test hypotheses related to ecological adaptation for arid environments (Barbour 1969; Barbour et al. 1974; Meinzer et al. 1990; Ogle and Reynolds 2002; Kropp and Ogle 2015). These studies have investigated the influence of diurnality, seasonal shifts, soil type, and neighboring plants on water use in *L. tridentata*, as well as differences in water use between *L. tridentata* and its South American relatives. For example, tetraploid *L. tridentata* had a much lower transpiration rate than the diploid South American *L. divaricata* Cav. (Barbour et al. 1974) under greenhouse conditions, and soil texture and composition played an important role in determining transpiration across seasons for tetraploid *L. tridentata* near Tucson, AZ (Ignace and Huxman 2009). Moreover, daily and seasonal fluctuations in temperature and moisture (Barker et al. 2006; Ogle et al. 2012), and the composition of neighboring plant communities (Kropp and Ogle 2015), strongly influenced transpiration for *L. tridentata* growing in the Mojave and Sonoran Deserts, while supplemental water and fertilization influenced water use for plants in the Mojave and Chihuahuan Deserts (Meinzer et al. 1988; Lajtha and Whitford 1989; Franco et al. 1994). However, these studies have typically been restricted to only diploids, or tetraploids, or hexaploids, leaving the relationship between water use and ploidy unclear despite the potential for relative water use to be a major contributor to fitness differences between cytotypes and the evolution of this important desert plant.

One exception to this single cytotype focus is an investigation by Barbour (1969) into the leaf wilting points for lab-grown seedlings of the three cytotypes collected from across the range. Barbour found that diploid seedlings wilted at a lower (more negative) water potential than the tetraploids or hexaploids,

counter to expectations of higher ploidies being better adapted for the more arid environments of the Sonoran and Mojave Deserts. In contrast, Ogle et al. (2012) conducted a study of stomatal conductance and transpiration for multiple common desert species across the Great Basin, Mojave, Sonoran, and Chihuahuan Deserts. *Larrea tridentata* in the Mojave Desert (probable hexaploids) was found to have lower transpiration than plants in the Chihuahuan Desert (probable diploids). Though these studies provide some evidence that ploidy influences water use in *L. tridentata*, the counterintuitive results and comparisons made between plants experiencing unique ecological conditions in disjunct ecoregions makes it difficult to interpret the role of genome duplication in facilitating ecological adaptation and the geographic expansion of *L. tridentata* into the many distinct habitats in which it is found.

In our controlled greenhouse experiment, we found that mean water use/total stomatal area did not differ significantly among diploids, tetraploids, and hexaploids (Fig. 3B). Despite the positive relationship between water use and stomatal area (Fig. 3A), the lack of a significant difference in water use/stomatal area may be due to a compensatory relationship between lower stomatal density with increased stomatal area. By pruning the plants to have approximately equal canopy sizes, we controlled for total leaf area among diploids, tetraploids, and hexaploids, resulting in similar total stomatal areas. Moreover, despite fluctuations in water use over the six weeks of the experiment, none of the cytotypes exhibited significantly different water use over time (Fig. 3C). Taken together, these results suggest that in areas of cytotypic contact, diploid, tetraploid, and hexaploid *L. tridentata* may have similar water use, and would be expected to have similar survival and fitness outcomes in the absence of other ecological interactions affecting water relations.

While these findings bring into question assertions that the cytotypes are differentially ecologically adapted (Yang 1967, 1970; Barbour 1969; Hunziker et al. 1977; Lewis 1980), we did not quantify water use efficiency, and it is unknown if the three cytotypes differ in biomass accumulation despite similar water use. There are also many other factors that can influence water use in natural settings that we did not examine here. In our study, we controlled for canopy size and stomatal density of the three cytotypes. However, naturally occurring cytotypic-specific differences in leaf and canopy architecture may influence relative stomatal densities and water use dynamics, altering fitness outcomes for competing naturally sympatric cytotypes (Laport and Ramsey 2015). For example, as shown here and by others (Barbour 1969; Barbour et al. 1977; Hunter et al. 2001), stomatal size and density vary with genome size in *L. tridentata*, and leaf and canopy size differs significantly between naturally sympatric cytotypes (Laport and Ramsey 2015). Thus, even in areas of

cytotypic contact subtle cytotypic-specific differences in stomatal size, density, and leaf area, have the potential to be major determinants of water use (Barbour et al. 1977; Kropp and Ogle 2015).

Diploids, tetraploids, and hexaploids also inhabit unique abiotic and biotic niche space that could influence water relations. Prior soil analyses and ecological niche models indicate the cytotypes occur in edaphically and climatically differentiated habitat (Laport and Minckley 2013; Laport et al. 2013), and community analyses indicate the cytotypes occur with distinct species associations in areas of contact (Laport et al. 2016). Differences in soil texture, nutrients, or co-occurring species could influence water relations over even relatively short distances. Such edaphic and climatic niche differences may also help structure the unique plant communities in which closely parapatric cytotypes occur (Laport et al. 2016). While the effect of differing inter-specific interactions on the three cytotypes in natural settings remains unknown, community structure has previously been shown to influence the water relations of *L. tridentata* (Kropp and Ogle 2015), and unique inter-specific interactions could influence the ability of the three cytotypes to accumulate water.

Complex cytotypic-specific interactions with the ecological setting are likely common. For example, Maherali et al. (2009) found that while diploid and tetraploid *C. angustifolium* had similar photosynthetic rates, indicating similar water use, tetraploids were able to maintain photosynthesis longer than diploids due to better soil moisture depletion. Moreover, this difference appears to only partially derive intrinsically from genome duplication, as artificially generated neotetraploids included in the study only partially recapitulated the physiological differences observed for natural tetraploids, consistent with postpolyploidization adaptation playing an important role in driving evolution in polyploid species (Husband et al. 2016). In *L. tridentata*, similar complex trait interactions may affect water relations. For example, soil nutrient or microbiota differences in the native soil attached to the roots of our wild-collected plants could have altered water use of the three cytotypes in our greenhouse study and should be investigated more generally for polyploid species (Powell and Doyle 2016; Segraves and Anneberg 2016). Moreover, Meinzer et al. (1990) found that epicuticular leaf resin was an important antitranspirant in *L. tridentata* and greater quantities of leaf resin among greenhouse-reared plants collected from a wild population in Nevada (probable hexaploids) were correlated with reduced stomatal conductance. Field and greenhouse observations suggest that tetraploids and hexaploids tend to more often have obviously resinous or hairy leaves (Barbour et al. 1977; Laport personal observation), and diploid plants have more often been observed succumbing to extreme drought conditions than polyploids when regular watering was inadvertently neglected (Laport personal observation). It remains unclear whether the

three cytotypes produce different amounts of epicuticular leaf resin or leaf hairs and whether this may have affected our results, but such observations suggest other traits, such as drought tolerance, may be more important for determining fitness or survival outcomes than water use as evaluated here (Yang 1967; Barbour 1969; Maherali et al. 2009).

Although our results do not support major differences in water use among diploid, tetraploid, and hexaploid *L. tridentata* when grown in a common environment under relatively mesic conditions, our observations do not preclude the possibility that complex abiotic, biotic, and genetic interactions play a significant role in determining cytotype occurrence and community structure in natural settings. We did not measure traits such as epicuticular resin or soil moisture depletion and drought tolerance, which may modulate water relations and survival independent of leaf area or stomatal attributes, and the traits we did measure were widely variable among cytotypes. This variability potentially reflects genetic variation harbored among our wild-collected experimental plants and suggests the sample sizes we employed in our greenhouse study may have been too small to detect subtle cytotypic differences in water use, especially if water use is a phenotypically plastic trait that is able to vary over a wide range of conditions. While differences in water use and other traits may exist between cytotypes, as suggested by prior field studies, additional investigations are required in a common environment to eliminate environmental and genotype- or cytotype-by-environment effects. Such investigations of inter-cytotype differences may reveal multi-character phenotypic differentiation among ploidy levels in *L. tridentata* informing our understanding of polyploid adaptation and evolution.

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SEED GERMINATION IN *VIOLA PEDUNCULATA* AND *VIOLA PURPUREA* SUBSP. *QUERCETORUM* (VIOLACEAE), CRITICAL FOOD PLANTS FOR TWO RARE BUTTERFLIES

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ABSTRACT

Viola L. are the larval food plants for a charismatic group of butterflies in *Speyeria* Scudder, 1872, which contains several rare and declining taxa in California and across North America. A current impediment to restoring *Viola* in California is a lack of knowledge of germination requirements. In this study we focused on determining conditions for successful germination of *V. pedunculata* Torr. & A. Gray, the food plant of the federally listed endangered *Speyeria callippe* (Boisduval, 1852) subsp. *callippe*, and *V. purpurea* Kellogg subsp. *quercetorum* (M.S. Baker & J.C. Clausen) R.J. Little, the food plant of the imperiled southern Coast Range butterfly, *S. adiate* (Edwards, 1864) subsp. *clemencei* (Comstock, 1925). We examined the effects of gibberellic acid, seed color, elaiosome removal, and duration of stratification on germination success. We found that both taxa germinate exceptionally well (73–100%) after a prolonged period of after-ripening and 6–10 wk of stratification. Gibberellic acid and elaiosome removal did not have strong effects on germination of *V. pedunculata*, and both dark and mottled *V. purpurea* subsp. *quercetorum* seeds germinated equally well. We make recommendations for successful lab germination of these two native violets, setting the stage for additional research to help restore their populations as well as their herbivores.

Key Words: Elaiosome, gibberellic acid, restoration ecology, *Speyeria adiate clemencei*, *Speyeria callippe callippe*, stratification.

Humans have been studying seed germination in native plants for thousands of years (Baskin and Baskin 1998). The majority of this effort has been focused on working with plants for use as agricultural or horticultural crops (Heiser 1990). One particularly relevant area for continued study of germination outside of these main uses is for conservation of native plants and the herbivores that depend on them. For example, sowing seeds or transplanting lab-grown seedlings may restore imperiled and endangered native plant populations, which, by extension, may conserve herbivore populations. This perspective motivated the current study of two *Viola* L. species that are larval food plants for declining butterflies.

Viola are generally herbaceous, zygomorphic plants with most species located in temperate climates. *Viola pedunculata* Torr. & A. Gray is endemic to California, found mainly in dry areas of full sun such as grassy slopes, hillsides, chaparral, and oak woodland (Baldwin et al. 2012). *Viola purpurea* Kellogg subsp. *quercetorum* (M.S. Baker & J.C. Clausen) R.J. Little is a native of California and southwest Oregon, found in areas of partial shade along dry slopes, generally associated with Yellow

Pine forest (Baldwin et al. 2012, The CalFlora Database 2016). *Viola pedunculata* is a myrmecochore that likely uses ballistic dispersal prior to ant dispersal (=diplochore, Bülow-Olsen 1984). The ants carry off seeds after being attracted to the small elaiosome. Elaiosomes are fleshy structures that contain lipids and proteins fed upon by the ants. Ballistic dispersal occurs once the capsule has opened and each valve desiccates to release the seeds. However, not all seed capsules disperse seeds explosively from the drying valves, and it is common to find open, dried valves containing seeds with elaiosomes (RIH, personal observation). *Viola purpurea* subsp. *quercetorum* is also very likely a diplochore with ballistic dispersal and small elaiosomes for attracting ants that aid in dispersal. Similar to *V. pedunculata*, not all capsules have explosive dispersal (RIH, unpublished data, personal observation).

Butterflies in the genus *Speyeria* Scudder, 1872 share a generally similar life history in which females lay eggs on and around dried remains of their larval food plant during summer with larvae diapausing through the remainder of summer and through winter (Scott 1979[81]). *Speyeria callippe* (Boisduval, 1852) subsp. *callippe* is a federally listed endangered butterfly species in California, limited to mainly grassland habitats (Black and Vaughan 2005) adjacent to San Pablo Bay and San Francisco Bay (United States Fish and Wildlife Service 2009). In spring when violets emerge, larvae break diapause and feed on their food plant, *V. pedunculata*, and

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FIG. 1. *Viola pedunculata* (left) and *V. purpurea* subsp. *quercetorum* (right, with *Erodium* L'Hér. in foreground), both from Chews Ridge, Monterey Co.

adults are on the wing from May through June (Black and Vaughan 2005, United States Fish and Wildlife Service 2009). *Speyeria adiastra* (Edwards, 1864) subsp. *clemencei* (Comstock, 1925) is endemic to the southern California coast range in Monterey Co. and San Luis Obispo Co. (Zaman et al. 2015). This species has the second most limited range in the genus; the southernmost subspecies *atossa* (Edwards, 1890) is extinct (Zaman et al. 2014). Females lay eggs in June and July on leaf litter and senescing leaves of their food plant, *V. purpurea* subsp. *quercetorum*. The larvae commence feeding the following spring (Zaman et al. 2014).

Very few populations remain of *S. c. callippe* (United States Fish and Wildlife Service 2009) and *S. a. clemencei* (Zaman et al. 2015). The fact that *S. c. callippe* and *S. a. clemencei* feed on specific violet taxa makes the presence and abundance of the food plant of critical importance for presence and persistence of the butterfly (United States Fish and Wildlife Service 2009, Zaman et al. 2014). A common hypothesis for the decline of *Speyeria* butterflies is habitat destruction causing a reduction in host plant populations (Hammond and McCorkle 1983). Restoration of food plants in populations where the butterflies are no longer present or declining is a promising way to manage and conserve *Speyeria* butterflies (Gehring et al. 2013). Two possibilities for restoring food plant populations are sowing lab-grown seeds or outplanting seedlings. Planting seeds in existing communities, with or without the focal species present, has generally been successful in increasing plant populations (Turnbull et al. 2000). For seed sowing to be effective, robust seeds with high survival rates must be produced. Outplanting has been shown to lead to better establishment (Buisson et al. 2006) and reduced vulnerability to pathogens and predators (Heithaus 1981, Ohkawara and Higashi 1994). However, effective outplanting requires maintenance to minimize stress during growth (Landis and Haase 2008), and a standard seedling size must be determined to increase the

likelihood of survival (Dumroese et al. 2016). With either approach restoration of larval food plant populations of declining California coast range *Speyeria* butterflies is currently hindered by lack of information on plant biology. Identifying conditions under which these native plants successfully germinate will facilitate their restoration.

To better understand the native larval food plants of *S. c. callippe* and *S. a. clemencei* and to lay the groundwork for future conservation of these butterflies, we examined the effects of duration of cool-wet conditions (i.e., stratification), gibberellic acid (GA, a growth hormone), seed color (assumed to correlate with seed quality), and elaiosome presence on germination success of *V. pedunculata* and *V. purpurea* subsp. *quercetorum*.

MATERIALS AND METHODS

Seed Sources and Characteristics

Viola pedunculata seeds were obtained from wild plants in Alameda Co., California, in June 2014. Specific sites where *V. pedunculata* seeds were collected were the Ohlone Conservation bank (37.55507, -121.76568) and nearby Wauhab Ridge (37.52755, -121.78098). These golden yellow (Fig. 1) perennial violets bloom from February to April (Munz and Keck 1968, The CalFlora Database 2016). The fruit capsules are glabrous and relatively large (5–11 mm; Baldwin et al. 2012). The seeds are 2.7 mm long and shiny dark brown to black (Munz and Keck 1968, Baldwin et al. 2012).

Viola purpurea subsp. *quercetorum* seeds were collected from wild plants at Chews Ridge, Monterey Co., CA (36.31336, -121.57323) in July 2014. *Viola purpurea* subsp. *quercetorum* is a perennial violet with lemon yellow flowers (Fig. 1) (Baldwin et al. 2012). The fruit is a pubescent capsule 4–12 mm long (Baldwin et al. 2012) containing <10 seeds (RIH personal observation). The seeds for *V. purpurea* subsp. *quercetorum* are dark brown, and Munz and Keck (1968) report seed length to be 2.7 mm. Our observations on dark brown seeds from a single capsule from Chews Ridge are similar (length \bar{x} = 2.74, SD = 0.03; width \bar{x} = 1.67, SD = 0.04, n = 5).

Seed characteristics, such as size and color, naturally vary, suggesting that not every seed has the same potential for germination (Agnieszka and Holubowicz 2008). Previous research has found *Viola* seed coloration to be an indicator of germination capacity (Agnieszka and Holubowicz 2008). Our collected seeds had natural variation in coloration, allowing us to sort the seeds into groups based on seed color and pattern (Fig. 2, and see below).

Experimental Design

Viola purpurea subsp. *quercetorum* and *V. pedunculata* seeds undergo a prolonged warm-dry period during the end of summer and early autumn (i.e.,

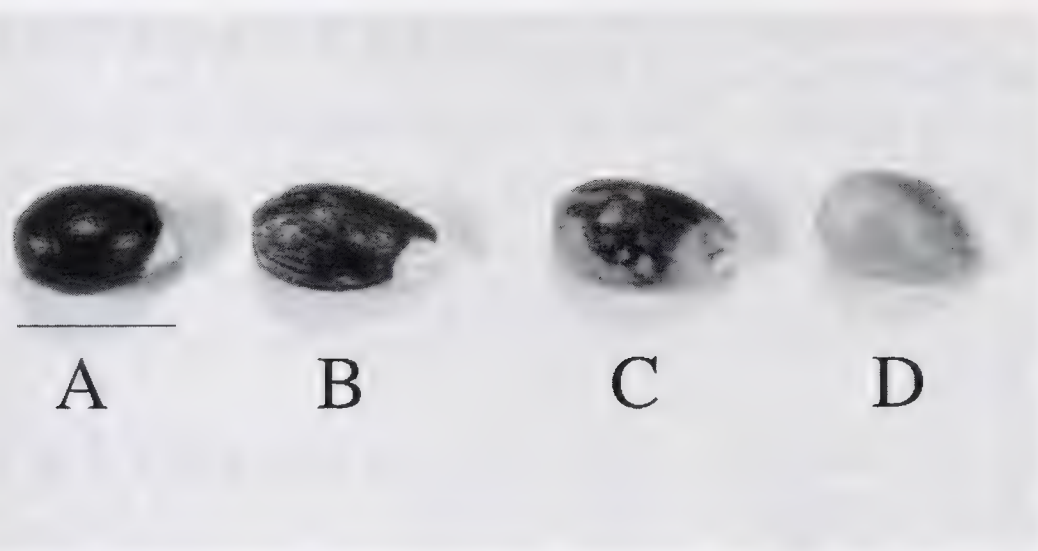


FIG. 2. Seed classes scored for *Viola pedunculata* (scale bar is 3 mm). From left to right, seed class A (large, all black), B (dark brown to black seeds with some mottling or streaking), C (brown seeds with obvious streaks/mottling), and D (pale or light brown seeds). Only class A seeds were used for this experiment.

after-ripening), followed by cool-wet winter conditions (i.e., stratification) before germinating in the spring. During these periods, the seeds must remain dormant in order to survive, suggesting that the wet winter period acts as a germination cue (Baskin and Baskin 1998). In order to approximate this natural cycle, seeds were placed first in a warm-dry period and then placed in stratification for varying durations (1, 5, or 10 wk). This range of stratification duration treatments was chosen to obtain good sample sizes with our limited seed numbers, and to investigate whether germination success varied with length of stratification.

An additional factor that may affect germination in diplochore species, such as *V. purpurea* subsp. *quercetorum* and *V. pedunculata*, is removal of the elaiosome by ants. Gehring et al. (2013) found that although not required for germination, elaiosome removal reduced seedling mortality. Thus to reduce the potential effect of fungal pathogens we removed elaiosomes from all seeds in Experiments 1 and 2. We directly tested the effect of elaiosome presence on germination in Experiment 3.

Finally, gibberellic acid, a naturally occurring growth hormone, is known to break dormancy in seeds (Paleg 1960). Gibberellic acid can increase germination rates and overall germination success in many species (Deno 1993, Zhang 1998, de Mello et al. 2009), although its effects may alter growth of the plant (Bachelard 1968). In an effort to find conditions optimal for germination, seeds were soaked in four different concentrations of gibberellic acid (see below).

Experiment 1: Effect of Gibberellic Acid and Stratification Duration on *Viola pedunculata*

Seeds were treated with a warm-dry, after-ripening treatment from time of collection in early June 2014, until 21 January 2015 (approx. 7 mo). During this time seeds were air dried in the lab in open plastic/wax paper bags near a window with the blinds closed. Temperature ranged from 20°C to 27°C. Prior to

stratification seeds were ranked to investigate the potential association of seed coat color and germination success. Large, all black seeds were classified A, dark brown to black seeds with some mottling or streaking were classified B, brown seeds with obvious streaks/mottling were classified C, and pale or light brown seeds were classified D (Fig. 2). Only class A seeds were used for this experiment. Groups of 30 seeds were exposed to one of three stratification lengths (1, 5, and 10 wk) and one of four concentrations of gibberellic acid, hereafter abbreviated GA (GA = gibberellic acid 3, $C_{19}H_{22}O_6$, 346.4 g/mol; Gold Biotechnology, St. Louis, MO) making a total of 12 different treatment combinations and 360 seeds tested. Gibberellic acid treatments consisted of a 22-hr pre-stratification soak in one of four concentrations of GA: 0 mg/liter (control), 100 mg/liter, 250 mg/liter, and 500 mg/liter. Elaiosomes were removed from all seeds prior to GA treatment to reduce fungal pathogens (Gehring et al. 2013). Prior to GA treatment, all seeds were held in a folded paper towel funnel and rinsed with 100 ml of 10% bleach, followed by three rinses of 100 ml deionized water.

For stratification, the groups of 30 seeds for each duration treatment were split into three groups of 10 and placed in folded autoclaved unbleached brown coffee filters. During stratification the seeds were placed in one of three autoclaved glass jars with one treatment level present in each glass jar for each stratification length. All glass jars were placed within the same foam cooler with lid, and the foam cooler was placed inside an Isotemp refrigerator (Thermo Fisher Scientific, Waltham, MA) set to 4°C. An Ibutton thermochron (Thermochron Corp., Milwaukee, WI) data logger placed within the foam cooler recorded an average temperature of 3.6°C (SD = 0.53°). Stratification was timed so that all seeds were moved to germination conditions on a benchtop in the lab at the same time (2 April 2015). Benchtop germination was done in autoclaved petri dishes with seeds placed between autoclaved filter paper moistened with deionized water. An Ibutton thermochron data logger placed on the benchtop near the germinating seeds recorded an average temperature of 22.3°C (SD = 0.46°). Seeds were checked weekly during stratification and germination periods and determined to have germinated when the radicle emerged.

Experiment 2: Effect of Seed Color and Stratification Duration on *Viola purpurea* subsp. *quercetorum*

Seeds were kept in warm-dry conditions as in Experiment 1 from July 2014 until 21 January 2015 (approx. 6 mo). Prior to stratification seeds were ranked to investigate the potential association of seed coat color and germination success. Large, all brown to black seeds were classified A, dark brown seeds with some mottling/streaking were classified B, and pale seeds classified C. Because we had few seeds for

TABLE 1. Results of Experiment 1 showing the effect of gibberellic acid (GA) and stratification duration (“weeks of stratification”) on germination of *Viola pedunculata*. Intercept is GA 0 mg/liter and 0 wk stratification.

	Coefficient	<i>z</i>	Pr (> <i>z</i>)
Intercept	−0.553	−1.46	0.144
GA	0.0113	4.61	<0.0001
Weeks of stratification	0.582	5.08	<0.0001
GA × weeks of stratification	−0.0018	−4.73	<0.0001

this species we tested class A and B seeds for all stratification duration levels, and C seeds were tested for 5 wk only. Seeds were not treated with gibberellic acid. Immediately before stratification, elaiosomes were removed and seeds were soaked for 22 hr in 20 ml deionized water. Seeds were soaked in batches of 14–20 seeds, which were split into two approximately equal groups and placed in a folded unbleached brown coffee filter for stratification for 1, 5, or 10 wk. For stratification, groups of seeds were placed in separate glass jars such that one replicate of each stratification level was together in each of two jars. All seeds were maintained during stratification in the same foam cooler with lid as in Experiment 1. Stratification was started for the 10-wk group first, followed by 5-wk, and finally 1-wk, in order for all seeds to start germination on the lab benchtop at the same time (2 April 2015). Lab benchtop conditions and setup were the same as in Experiment 1. Seeds were checked weekly during stratification and germination periods and determined to have germinated when the radicle emerged.

Experiment 3: Effect of Elaiosome Removal on *Viola pedunculata*

Seeds were classified by color, and treated to a warm-dry period as in Experiment 1. Only seeds of class B were used for this experiment because sufficient amounts of B seeds were available. Eight groups of 13–15 seeds had their elaiosomes removed, and seven groups of 13–15 seeds each were left with their elaiosome intact. Prior to stratification, each group of seeds was soaked for 22 hr in 20 ml deionized water, followed by a rinse with 100 ml of 10% bleach, followed by three rinses of 100 ml deionized water. Stratification conditions and setup were the same as in Experiment 1. Seeds were checked weekly for an emergent radicle during each of 10 wk of stratification at which point observations ended.

Statistical Analysis

For each experiment we used a generalized linear model to evaluate the effect of the predictor variables (weeks of stratification, GA concentration, seed class, elaiosome presence), on total germination success. We calculated total germination success for each batch of seeds as the number of plants that successfully

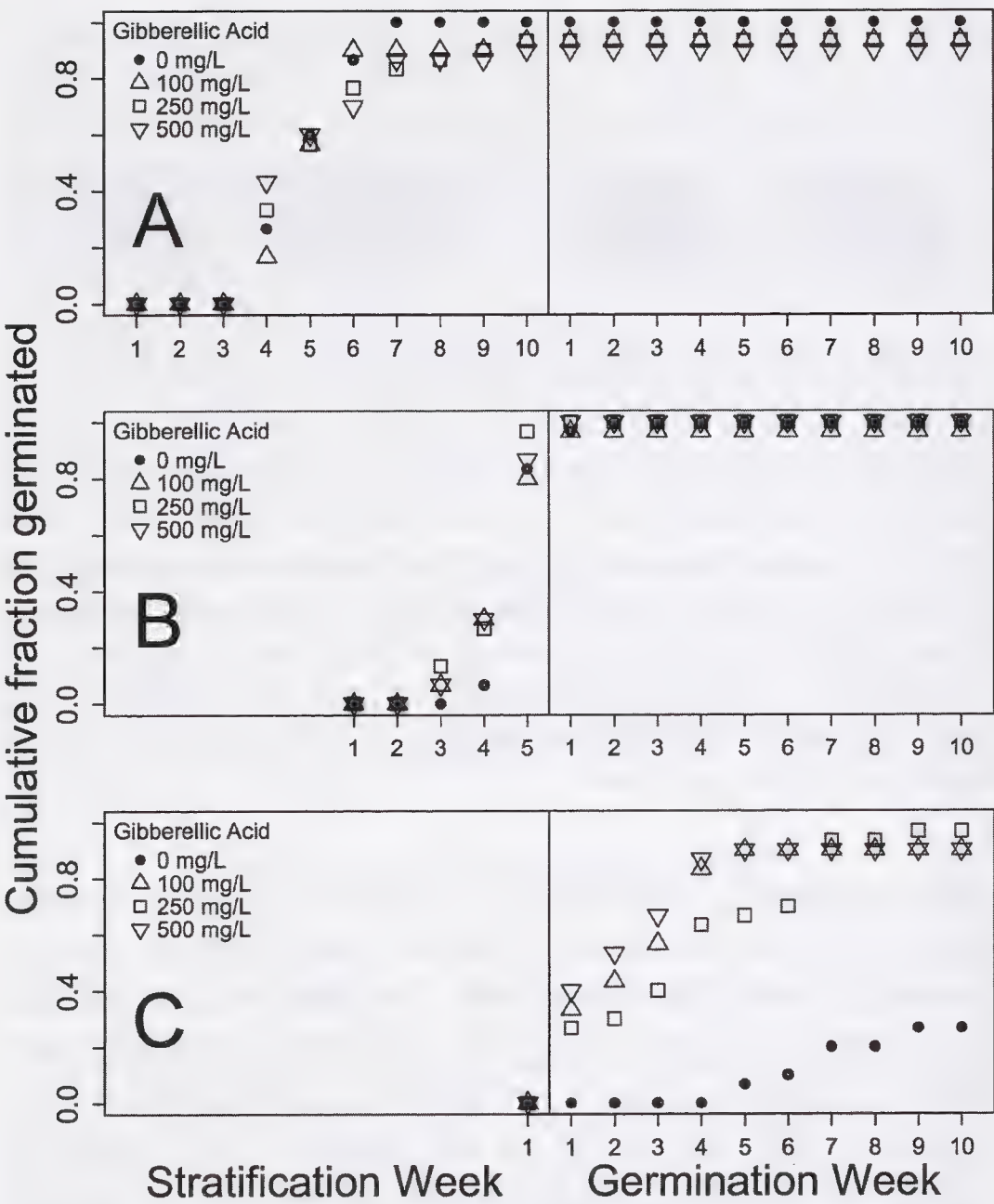


FIG. 3. Cumulative fraction of *Viola pedunculata* seeds germinated across different concentrations of gibberellic acid and stratification duration treatments. The temporal sequence of the experiment is shown with weeks of stratification on the left followed by germination on the right. Panel A shows 10-wk stratification treatment, panels B and C show 5-wk, and 1-wk stratification treatments, respectively.

germinated at the end of the entire experiment. Interaction terms between weeks of stratification and GA concentration for *V. pedunculata*, or weeks of stratification and seed class for *V. purpurea* subsp. *quercetorum* (A and B only) were included in the models. For Experiment 2, a separate model was fitted for the 5-wk treatment in order to test the effect of seed class across all three levels (A, B, C), since class C seeds were only available for the 5-wk treatment. Because the response variable was binomial (success/failure), we used the binomial family with logit link function. Analyses and graphs were done in R 3.1.2 (R Development Core Team 2014).

RESULTS

Experiment 1: Effect of Gibberellic Acid and Stratification Duration on *V. pedunculata*

Experimental procedures here resulted in high germination success in *V. pedunculata* seeds. Weeks of stratification had a strong positive effect on germination success ($z = 5.08$, $P < 0.0001$) (Table 1, Fig. 3) and including weeks of stratification in the model was a significant improvement (residual deviance for weeks of stratification = 83.8, $df = 33$, $P < 0.0001$). Seeds in the 10-wk and 5-wk

TABLE 2. Results of Experiment 2 showing the effect of seed class (A and B only) and stratification duration (“weeks of stratification”) on germination of *Viola purpurea* subsp. *quercetorum*. Intercept is class A seeds and 0 wk stratification.

	Coefficient	<i>z</i>	Pr (> <i>z</i>)
Intercept	−3.39	−3	0.0027
Class B seeds	−22.63	−0.004	0.997
Weeks of stratification	0.769	3.27	0.0011
Class B seeds × weeks of stratification	4.73	0.004	0.997

stratification treatments started germinating during stratification beginning in week 3–4, and reached 100% germination success in experimental week seven (Fig. 3A and 3B). In the 1-wk stratification treatment, no seeds germinated during stratification and total germination success was only 27% without GA (Fig. 3C).

Gibberellic acid did have a significant effect on germination success ($z = 4.61$, $P < 0.0001$, Table 1), but it was relatively weak given the coefficients and the nature of the significant interaction with weeks of stratification ($z = -4.73$, $P < 0.0001$). Including both GA and the interaction of GA × weeks of stratification in the model significantly improved fit (residual deviance for GA = 105.9, $df = 34$, $P < 0.0001$, residual deviance for GA × weeks of stratification = 60.2, $df = 32$, $P < 0.0001$). The coefficient for GA × weeks of stratification was negative, indicating that as weeks of stratification increases, the effect of GA decreases (Table 1). Figure 3 shows that GA did not have a significant impact on germination success in the 10- and 5-wk treatments, where seeds not treated with GA did just as well as those that were treated with the hormone (Fig. 3A and 3B). In the 1-wk stratification treatment, GA was necessary to achieve the high germination rates (>90%) seen in the longer stratification treatments. Otherwise, within each week of the 5- and 10-wk stratification treatments, there was no consistent relation between the higher concentrations of GA and higher germination success (Fig. 3).

Experiment 2: Effect of Seed Color and Stratification Duration on *V. purpurea* subsp. *quercetorum*

Experimental procedures here resulted in high germination success in *V. purpurea* subsp. *quercetorum* in nearly all treatment combinations. Weeks of stratification and seed class both affected probability of germination for *V. purpurea* subsp. *quercetorum*. Weeks of stratification had a very strong and significant positive effect on *V. purpurea* subsp. *quercetorum* seed germination success (Table 2, $z = 3.27$, $P = 0.0011$), and including weeks of stratification significantly improved the fit of the model (residual deviance of weeks of stratification 10.7, $df = 9$, $P < 0.0001$). Final germination success was 73–100% in class A and B seeds for the 5- and 10-wk stratification treatments (Fig. 4). In the 10-wk stratification treatment the A and B seeds reached their maximum

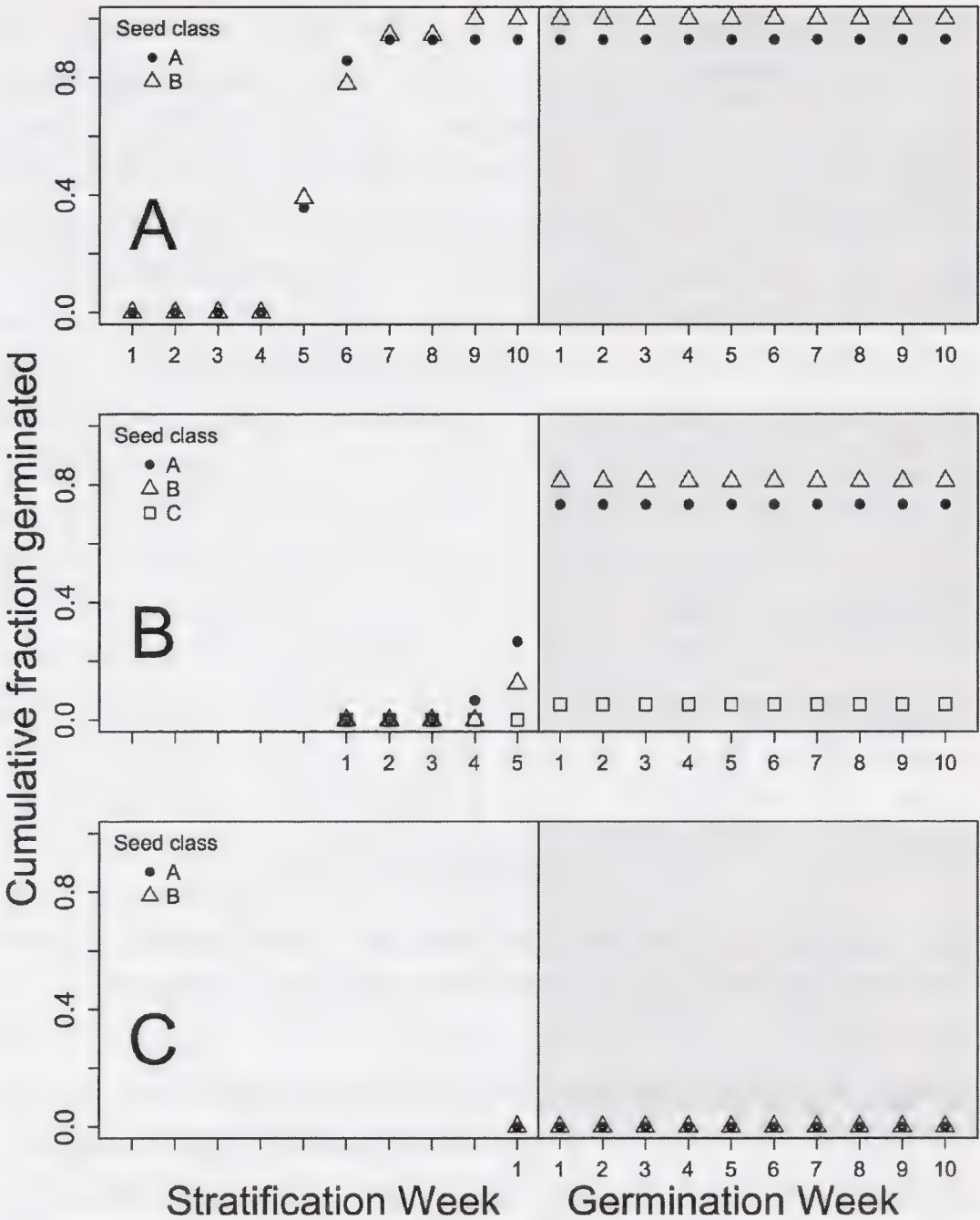


FIG. 4. Cumulative fraction of *Viola purpurea* subsp. *quercetorum* seeds germinated for three seed classes and stratification duration treatments. The temporal sequence of the experiment is shown with weeks of stratification on the left followed by germination on the right (gray). Class A was assumed a priori to be highest quality, with C lowest quality. Panel A shows 10-wk stratification treatment, panels B and C show 5-wk and 1-wk stratification treatments, respectively.

germination rate by week 9 of stratification (90% and 100% respectively), with no increase during the germination period. In the 5-wk stratification, the A and B seeds began germinating in weeks 4 and 5, and reached the final cumulative percent germination 1 wk later during the germination period (73% and 81% respectively). There was no germination for class A and B seeds in the 1-wk stratification treatment (0 %; Fig. 4C). After the 10-wk germination period ended, we continued observations on the remaining A and B, 5-wk and 1-wk treatment seeds. We placed these seeds back into stratification for an additional 14 wk, at which point the 5-wk A and B seeds reached 100% final germination, and the 1-wk A and B seeds reached ~50% final germination.

Seed class was a significant predictor of germination success; however, this was only because of the very poor germination in class C seeds. For the model that included all stratification levels and only class A and B seeds, germination success of B seeds did not differ from the null model with A seeds ($z = -0.004$, $P = 0.997$), and A and B seeds did not differ across weeks given that there was no interaction with weeks of stratification ($z = 0.004$, $P = 0.997$). Furthermore, including seed class did not improve

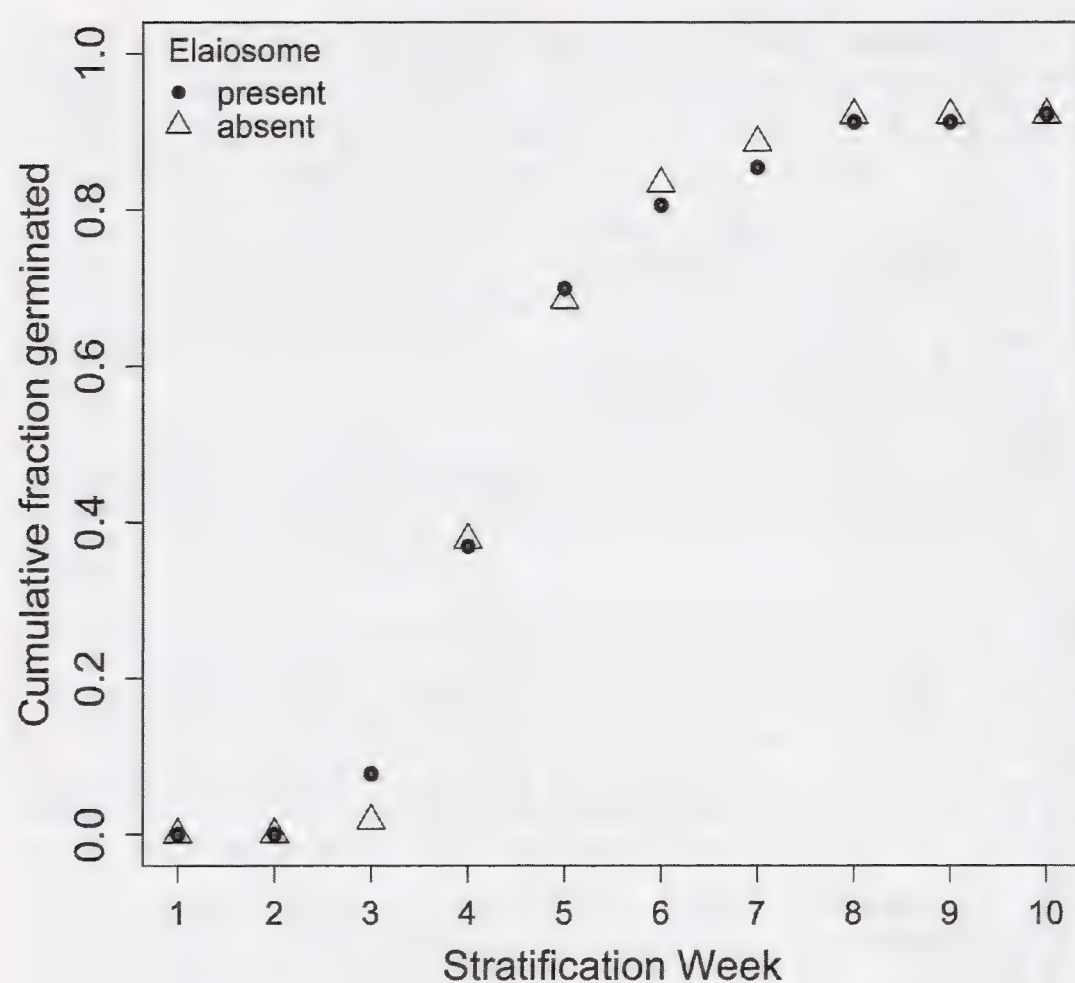


FIG. 5. Cumulative fraction of germinated *Viola pedunculata* seeds with elaiosome intact and removed.

model fit (residual deviance of seed class = 89.95, $df = 10$, $P = 0.46$). When comparing seed classes directly in the 5-wk stratification treatment, class A and B seeds did not differ in germination success ($z = 0.525$, $P = 0.60$), and there was significantly lower germination success in the class C seeds (5.3% total germination, $z = -3.3$, $P < 0.0001$).

Experiment 3: Effect of Elaiosome Removal on *V. pedunculata*

Removing elaiosomes did not alter germination success ($z = 0.035$, $P = 0.97$), and the model including elaiosome removal was not a significant improvement over the null model (residual deviance No elaiosome = 18.04, $df = 13$, $P = 0.97$). Germination success reached a final germination rate of 92% for both groups (Fig. 5). The class B seeds examined in this experiment began germinating in week three and four and reached the maximum percent germinated by week seven and eight (Fig. 5). This was similar to the 10-wk stratification treatment with class A *V. pedunculata* seeds and no gibberellic acid (Fig. 3A, 0 mg/liter curve), although that group reached 100% germination before the end of stratification, compared with 92%.

DISCUSSION

Results of this study demonstrate that both *Viola pedunculata* and *Viola purpurea* subsp. *quercetorum* can be successfully germinated when exposed to a warm-dry period followed by a cool-wet period. These species appear to be very amenable to germination given that the germination rates observed here are among the highest for *Viola* spp. (Deno 1993, Barekat et al. 2013, Gehring et al. 2013). We were able to achieve 90–100% germination rates for seeds of both *V. pedunculata* and *V. purpurea* subsp. *quercetorum* after several months of dry

storage and several weeks of stratification in dark wet conditions with or without elaiosomes, and GA was not necessary.

The germination rates observed for this study were higher than those obtained for other *Viola*, indicating that experimenting with dormancy conditions and duration to match natural conditions could increase germination success. Deno (1993) reported results for 23 *Viola* spp. and found maximum percent germination rates of 85–94% for four taxa (*V. cucullata* Aiton, *V. dactyloides* Roem. & Schult., *V. fimbriatula* Sm., *V. incisa* Turcz.), germination rates closer to 70% for four additional species (*V. altaica* Ker Gawl., *V. appalachensis* L.K. Henry, *V. papilionacea* Pursh, *V. tricolor* L.), but with the majority of results well below this percentage, even for the species with high rates under some conditions. Summarizing, Deno (1993, p. 229) stated, “at least in some species germination requires light and/or GA-3”. Although neither was required for taxa in our study, light and GA should be considered for additional species. Deno (1993) also noted that dry storage is not tolerated well in *Viola*, “...except for *V. rugulosa*, a prairie species”, and “[p]ossibly other prairie and dry land species”. Natural tolerance to extended dry conditions may explain the relatively high germination rates in the taxa studied here. *Viola pedunculata* and *V. purpurea* subsp. *quercetorum* both inhabit the Southern Coast Ranges of California, which have prolonged dry periods in summer and autumn. In a recent study of *V. pedata* L., a prairie species, the duration of warm-dry conditions was associated with increased germination rate (Gehring et al. 2013). In that study, the maximum germination rates of 50–60% were obtained using warm-dry periods of up to 12 wk that cycled daily to match summer conditions (Gehring et al. 2013). Perhaps germination could be increased with extended warm-dry conditions in this and other prairie species.

Viola pedunculata germinated very well under conditions that roughly match the natural cycle of the species. Addition of GA did not increase overall germination success or change germination rate, except in the 1-wk stratification treatment (Fig. 3C), which would not likely have germinated at all without exposure to GA. Thus, although GA helped germination with a short stratification period (Fig. 3C), it is unnecessary. Exposing seeds to an extended cool-wet, winter-like period, appears to be sufficient and very effective, and likely avoids increased amounts of fungal growth (see below).

Viola purpurea subsp. *quercetorum* also germinated very well under conditions that roughly match the natural cycle of the species. The extended cool and wet conditions of the 10-wk stratification may have increased germination relative to the 5-wk stratification (see below). Seed class was a determinant of germination success, with A and B class seeds germinating much better than class C seeds, indicating that the pale color of seeds is strongly associated with reduced viability. The lack of difference between

A and B seeds indicates that both completely dark seeds as well as seeds with extensive mottling are viable. However it is unknown what ramifications, if any, seed color has for survival in nature in these species. Potential explanations could include reduced predation as a result of crypsis on the ground (Porter 2013) or by resembling pale nonviable seeds that are not of interest to predators (Myczko et al. 2015).

In terms of achieving germination, the addition of GA and removal of elaiosomes were unnecessary and may negatively affect seeds or seedlings. Gibberellic acid was only useful at increasing germination rates in *V. pedunculata* when a short stratification period was used, and so may be useful if quick germination is required without cool conditions. However, exposing the seeds to wet but warm conditions increased fungal growth and led to seedling mortality. Using a longer stratification time would avoid this and achieve similar or better germination rates. In fact, *V. pedunculata* class A and B seeds from the 5-wk stratification treatment which showed 73% and 81% success respectively, reached 100% success when put back into stratification after the entire 10-wk germination period on the benchtop. Gibberellic acid did not appear to alter growth form of planted seedlings.

Elaiosome removal is not required for germination, but it may reduce or eliminate mortality due to fungal pathogens. Elaiosome removal did not affect germination success in *V. pedunculata* (Fig. 5), but elaiosomes appeared associated with fungal growth as in other studies (Gehring et al. 2013). We observed little to no mold during stratification, but it was widespread during the germination period, and in planted seedlings (i.e., damping off). Although damping off occurred in planted seedlings, it is unknown at this point what effect mold has on the long-term viability of these two species. Subsequent research could test methods for reducing the susceptibility of seeds/seedlings to fungal pathogens.

The differences in results for *V. pedunculata* and *V. purpurea* subsp. *quercetorum* obtained here are likely the result of local adaptation to environmental conditions, and as mentioned above, experimenting with duration of stratification and after-ripening may increase germination success. The *V. pedunculata* seeds used in this study were from a warmer, lower elevation site than those of *V. purpurea* subsp. *quercetorum*, and *V. pedunculata* is found at lower elevations (Baldwin et al. 2012) and in drier, warmer microhabitats where they are sympatric. Seeds of *V. purpurea* subsp. *quercetorum* from Chews Ridge regularly experience snow and colder temperatures. Corresponding to this difference in environment, seeds of *V. pedunculata* reached maximum germination success slightly before *V. purpurea* subsp. *quercetorum* (Fig. 3) under the same conditions in our 10-wk stratification treatments. In the 5-wk treatments the two species reached maximum germination success at about the same time. However, the 5-wk stratification did not appear to be as successful a stratification treatment for *V. purpurea* subsp.

quercetorum. This indicates that exposure to longer cool-wet, winter-like conditions can increase germination success in *V. purpurea* subsp. *quercetorum* because they better simulate natural conditions. Interestingly, for both species the 5-wk stratification treatment appeared to reach maximum germination sooner (1–2 wk) than in the 10-wk treatment (Fig. 3). This is not likely because of the shorter stratification time, but rather because of the extended time these seeds spent in the warm-dry conditions. This is corroborated by the fact that some leftover *V. pedunculata* seeds collected at the same time (June 2014) as those used in this experiment were put into stratification in February/March 2016, 20–21 mo after being collected, and began germinating in 1–3 wk, which was 2–3 wk earlier than they began germinating the previous year. Together this indicates it may be worth experimenting with a longer, but appropriate warm-dry period.

Based on the results here we can make the following recommendations for improving germination success for *V. pedunculata* and *V. purpurea* subsp. *quercetorum*. All of the seeds that germinated during the 10-wk stratification (Figs. 3A and 4A), and nearly all that germinated in the 5-wk stratification treatment (Figs. 3B and 4B), did so during the stratification period, indicating the germination period was not necessary and should be avoided to decrease fungal growth at warmer temperatures. For both species a prolonged warm-dry period of approximately 7 mo should be followed by exposure to 6–10 wk of stratification in the dark. Since any restoration will likely involve using seeds from the same, or a nearby population (i.e., the same ecotype), adjusting the duration of warm-dry and cool-wet treatments to match that of the particular seed source will likely maximize germination success.

The results presented here make restoration of *V. pedunculata* and *V. purpurea* subsp. *quercetorum* populations seem promising, but additional studies are needed. The methods outlined here are very useful as a first step toward efficiently generating greenhouse populations of these species that could be used in outplanting or seed sowing. Future work on *V. pedunculata* and *V. purpurea* subsp. *quercetorum* should be aimed at growing lab-germinated seedlings to better understand conditions for propagating these species. In particular, decreasing damping off and achieving conditions that promote vigorous flowering and fruiting are important. Damping off can be reduced by avoiding excessively wet conditions. In addition, an antifungal treatment may be applied. Finally, research on the effectiveness of sowing seeds and outplanting methods is needed. If these methods can enhance or restore *V. pedunculata* and *V. purpurea* subsp. *quercetorum* populations, they may help in reintroducing *S. callippe callippe* or *S. adiate clemencei* butterflies to restored areas or vacant habitat patches, as has been undertaken in other *Speyeria* species (Shepherd and Debinski 2005, Crone et al. 2007).

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A NEW SPECIES OF *LOESELIASTRUM* (POLEMONIACEAE) FROM NORTHERN ARIZONA

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ABSTRACT

Loeseliastrum franciscanum R. Crawford, a local endemic known from three populations in the eastern San Francisco volcanic field of Coconino County, northern Arizona is newly described and illustrated here. This species is similar to *L. depressum* (M. E. Jones ex A. Gray) J. M. Porter & L. A. Johnson, but is distinguished by its strongly bilabiate corolla, long exserted stamens, pinnatifid leaves, and mostly tridentate inflorescence bracts. A revised key to *Loeseliastrum* is presented.

Key Words: Endemic, *Loeseliastrum*, northern Arizona, Polemoniaceae, taxonomy.

Loeseliastrum (Brand) Timbrook (Polemoniaceae) is a genus of desert annuals characterized by bilaterally symmetric flowers, terminal compact inflorescences, stamens unequally inserted at the sinuses, and dentate leaves with each lobe possessing a single bristle. The genus is comprised of three species distributed across the Sonoran, Mojave, and Great Basin Deserts. Prior to segregation into *Loeseliastrum* (Timbrook 1986), species were included in *Langloisia* Greene (Brand 1907, Grant 1959). The original circumscription of the genus (Timbrook 1986) included two taxa, *Loeseliastrum matthewsii* (A. Gray) Timbrook and *L. schottii* (Torr.) Timbrook. Recent studies have since transferred *Ipomopsis depressa* M. E. Jones ex A. Gray into the genus (as *Loeseliastrum depressum* [M. E. Jones ex A. Gray] J. M. Porter & L. A. Johnson), based on morphological and molecular data (Porter and Johnson 2000, Porter et al. 2010).

In the spring of 2012 the first author collected specimens of *Loeseliastrum* with blue pollen, elongated inflorescences, and short bristles that represented an undescribed species. The specimens were collected in the Navajo Nation in Hopi Trail Canyon (near Cameron, Arizona) as part of a floristic inventory of the Little Colorado River Gorge. Subsequent searches of specimens in Arizona herbaria revealed two previous collections of this taxon from the vicinity of Wupatki National Monument. Greg Goodwin documented two additional localities in 2014, collecting and photographing the species in situ from sites on the CO Bar Ranch near Grey Mountain, Arizona.

TAXONOMIC TREATMENT

Loeseliastrum franciscanum R. Crawford sp. nov. (Figs. 1–3)—Type: USA, AZ, Coconino Co., Wupatki National Monument, Black Bottom Crater, 35°24'20.5920"N, 111°24'10.8366"W, 1769 m, 6 June 2015, R. Crawford 1440 (holotype: ASC; isotypes: ASU, ARIZ, DES, BRY, NY, RSA).

Loeseliastrum franciscanum resembles *L. depressum* by sharing stems that freely branch from the

base, short terminal bristles on the leaf lobes, small corollas (< 9 mm), and thin membranous rotund valves on the capsule. *Loeseliastrum franciscanum* differs from *L. depressum* by possessing pinnately lobed leaves, mostly tridentate inflorescence bracts, and a strongly bilabiate corolla with long exserted stamens.

Annual herbs, 2.5–17 cm tall, freely branching from the base, stems one to many, prostrate to erect, purple to green, sparsely to densely hirtellous with a mix of eglandular and gland-tipped hairs and longer flattened multicellular hairs (0.1–1 mm), more dense distally. Leaves basal and cauline, 5–20 × 0.1–7 mm wide, alternate, sessile to subsessile, entire to pinnately 3–5 lobed or toothed, each lobe or tooth with a single short terminal bristle, blades sparsely short hispid, with a mix of short eglandular and gland-tipped hairs and longer flattened multicellular trichomes. Inflorescence subcapitate, elongating in fruit, subtending bracts 2.5–10 mm long, divided distally into 3–5 teeth, ciliate below teeth; flowers 5–25, 5.8–7.5 mm long; calyx 2.9–4 mm long, accrescent, to 3–6 mm in fruit, lobes acuminate and scarious margined, sometimes with maroon streaks, subequal, bristle-tipped, glandular pubescent and with flattened multicellular hairs (0.1–1 mm), the wide intercostal hyaline membrane remaining intact throughout development; corolla white, funnelform, tube 2–3 mm long, limb bilabiate, upper lip 3-lobed, 2–3.5 mm long, with one or two yellow spots at the base of each lobe, usually outlined by lavender arches, the lower lip 2-lobed, 2–2.5 mm long, markings often faint or absent, lobes rounded to apiculate, sinuses unequal; stamens 5, ± equal, filaments 3.5–5 mm long, unequally attached at sinuses, strongly exserted and curved, anthers blue or white, pollen blue; style 3–4 mm long, exserted, stigma lobes 0.3 mm long. Fruit a capsule, 3-lobed in cross-section, 3–4 mm long, dehiscent at maturity via apical valves, the valves rotund, apiculate, translucent; seeds 7–12 per capsule, 1.3–1.8 mm long, olive to brown, reticulate and rugulose.



FIG. 1. Holotype (ASC1120555).

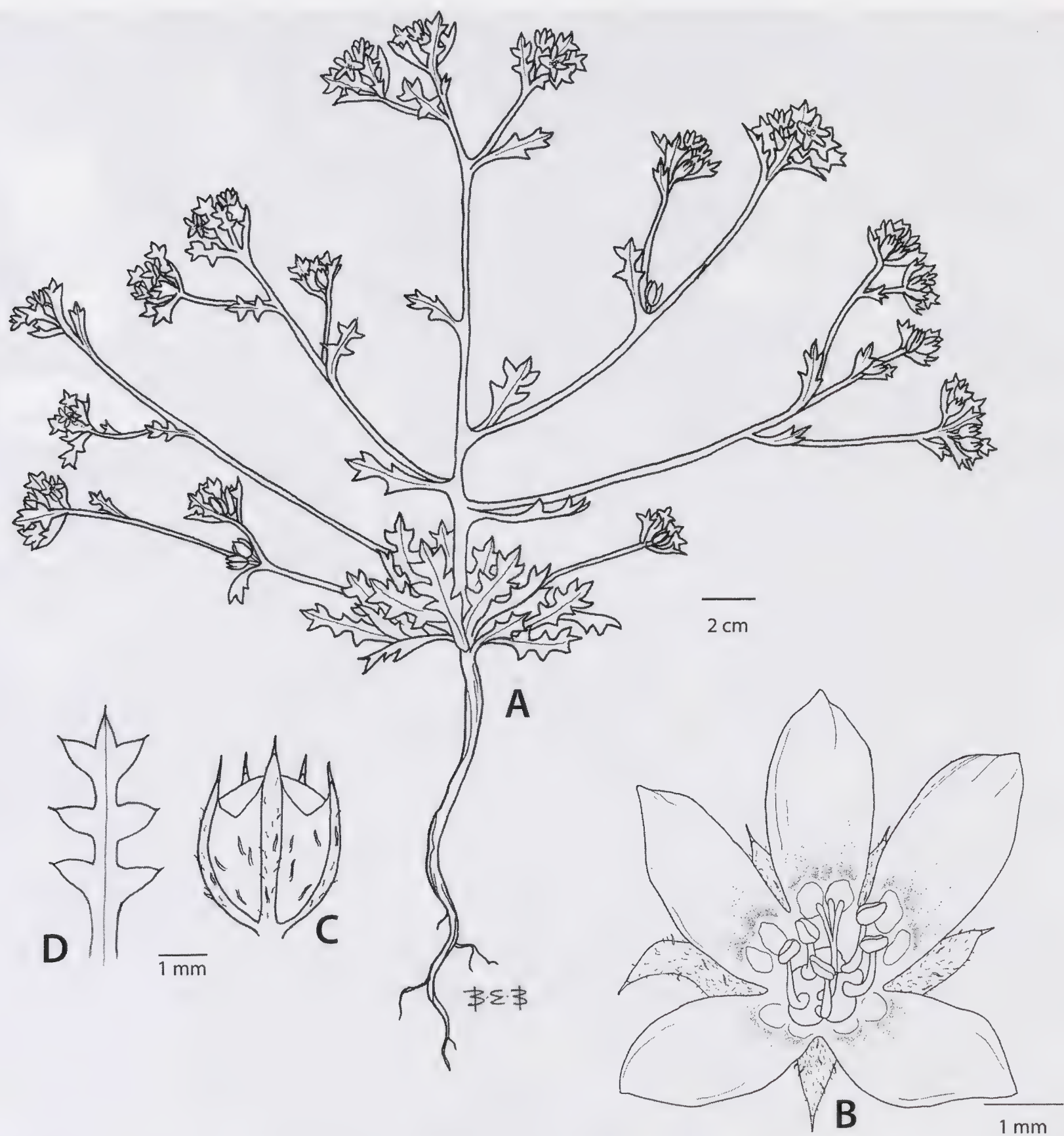


FIG. 2. Illustration of *Loeseliastrum franciscanum* from holotype (ASC1120555). A. Habit. B. Flower. C. Fruit. D. Leaf.

Paratypes: USA, NAVAJO NATION: Hopi Trail Canyon, 35°56'34.8144"N, 111°28'46.0232"W, 1323 m, 28 May 2012, *R. Crawford* 481 (ASC, NAVA); same location, 28 April 2013, *R. Crawford* 911 (ASC, NAVA, ARIZ, UVSC, SJNM); USA, ARIZONA, Coconino Co.: Wupatki National Monument, 12 May 1939, *D. Jones* 21 (ARIZ); Wupatki National Monument, Black Bottom Crater, 35°24'19.8036"N, 111°24'13.2512"W, 768 m, 9 June 2005, *K. Christie* 629 (ASC); same location, 09 May 2013, *R. Crawford* 976 (ASC, ASU, UNLV, UT, BRY, RENO-V, UCR); CO Bar Ranch, 35°40.117'N, 111°31.047'W, 1585 m, 10 May 2014, *G. Goodwin* 4527 (ASC, US); CO Bar Ranch, 35°41.450'N, 111°32.164'W, 1554 m, 25 Aug 2014, *G. Goodwin* 4602 (ASC).

ETYMOLOGY

Loeseliastrum franciscanum is named for its endemism to the eastern San Francisco Volcanic Field.

TAXONOMIC RELATIONSHIPS

We compared specimens of *L. franciscanum* to the collections available in the Deaver Herbarium (ASC),

which included four specimens of *L. depressum* from California and Nevada; 11 specimens of *L. schottii* (Torr.) Timbrook from Arizona, California, and Nevada, and five specimens of *L. matthewsii* (A. Gray) Timbrook from California (Appendix 1, Table 1). In addition, we examined digital images of type specimens for each species available at JSTOR Global Plants (<https://plants.jstor.org/>). The pinnatifid leaves, divided corolla, strong bilateral symmetry with long, exserted, recurved filaments suggests that *L. franciscanum* is morphologically similar to *L. matthewsii* and *L. schottii*. However, the combined suite of habit, floral, fruit, and vegetative characters support a closer relationship to *L. depressum* (Table 1). Both *L. franciscanum* and *L. depressum* can possess blue pollen (though not exclusively blue for *L. depressum*), hyaline calyx membranes that are wider than the ribs and remain intact throughout fruit development, thin translucent rotund valves, and large rugulose seeds. *Loeseliastrum franciscanum* and *L. depressum* share several vegetative traits including stems that freely branch from the base, bristles that are shorter than the leaf lobes, and cauline leaves with margins entire or 1–5 lobes. The calyx of *L. schottii* and *L. matthewsii* has a thin hyaline membrane that shatters early in fruit

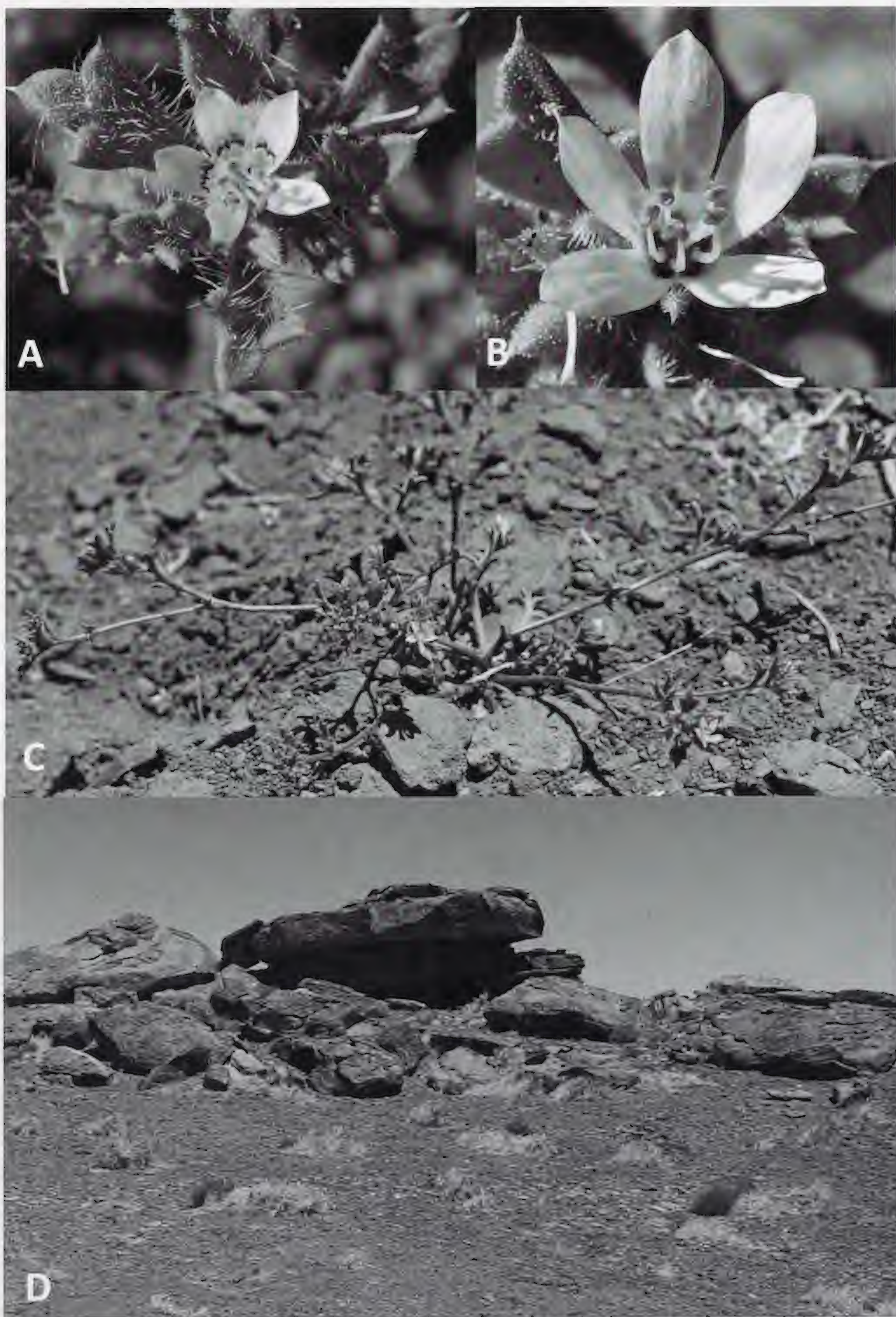


FIG. 3. Field photographs of *Loeseliastrum franciscanum*. A. Typical corolla, with yellow spots outlined with lavender arches at base of lobes. B. Atypical appearance of corolla lacking defining lavender arches. C. Habit freely branching from the base. D. Habitat at CO Bar Ranch, a gentle slope with moderate disturbance and clay soil derived from Moenkopi Formation. Photographs A, C, D by Greg Goodwin; photograph B by Rich Crawford.

TABLE 1. Comparison of morphological and ecological traits between *Loeselistrum* species.

	<i>L. franciscanum</i>	<i>L. depressum</i>	<i>L. schottii</i>	<i>L. matthewsii</i>
Stems	2–25 cm	2–12 cm	2–12 cm	3–15 cm
Lobes on largest leaves	Absent or poorly developed proximally, well-developed and numerous distally	Absent or poorly developed, when present concentrated distally	Well-developed throughout, equally spaced	Well-developed throughout, equally spaced
Lobe-to-bristle l ength	Leaf lobe > leaf bristle	Leaf lobe > leaf bristle	Leaf lobe < leaf bristle	Leaf lobe < leaf bristle
Inflorescence	Axillary and terminal, much elongated in fruit	Axillary and terminal, slightly elongated in fruit	Compact, terminal, not elongated in fruit	Compact, terminal, not elongated in fruit
Inflorescence bracts	3–5 distal teeth	Mostly entire or 1–2 very small teeth	3–5 distal teeth	3–5 distal teeth
Calyx	Hyaline membrane wider than ribs, remaining intact throughout fruit development	Hyaline membrane wider than ribs, remaining intact throughout fruit development	Hyaline membrane narrower than ribs, rupturing early in fruit development	Hyaline membrane narrower than ribs, rupturing early in fruit development
Calyx lobes	Bristles < 1 mm	Bristles < 1 mm	Bristles > 1.5 mm	Bristles > 1.5 mm
Corolla	4.3–7 mm, strongly bilabiate	5–8 mm, weakly bilabiate	8–15 mm, strongly bilabiate	11–21 mm, strongly bilabiate
Corolla, upper lip	2–3 mm	1–2 mm	3–7 mm	5–11 mm
Anthers	Long-exserted	Included	Long-exserted	Long-exserted
Pollen	Blue	Blue, yellow, or white	Yellow	Yellow
Capsule valves	Fully disarticulating tardily, thin, translucent, rotund	Fully disarticulating tardily, thin, translucent, rotund	Fully disarticulating at maturity, walls thick, opaque, lanceolate	Fully disarticulating at maturity, walls thick, opaque, lanceolate
Seeds	1.3–1.8 mm rugulose and finely textured	1.2–1.4 mm rugulose and reticulate	1 mm, flattened to rounded faces, finely textured	1 mm, flattened to rounded faces, finely textured
Distribution	Southern Colorado Plateau	Southwest Great Basin Desert, Northern Mojave	Southwest Great Basin Desert, Mojave and Sonoran Deserts	Mojave and lower Sonoran Desert
Flowering period	April–August	May–November	March–June	March–July

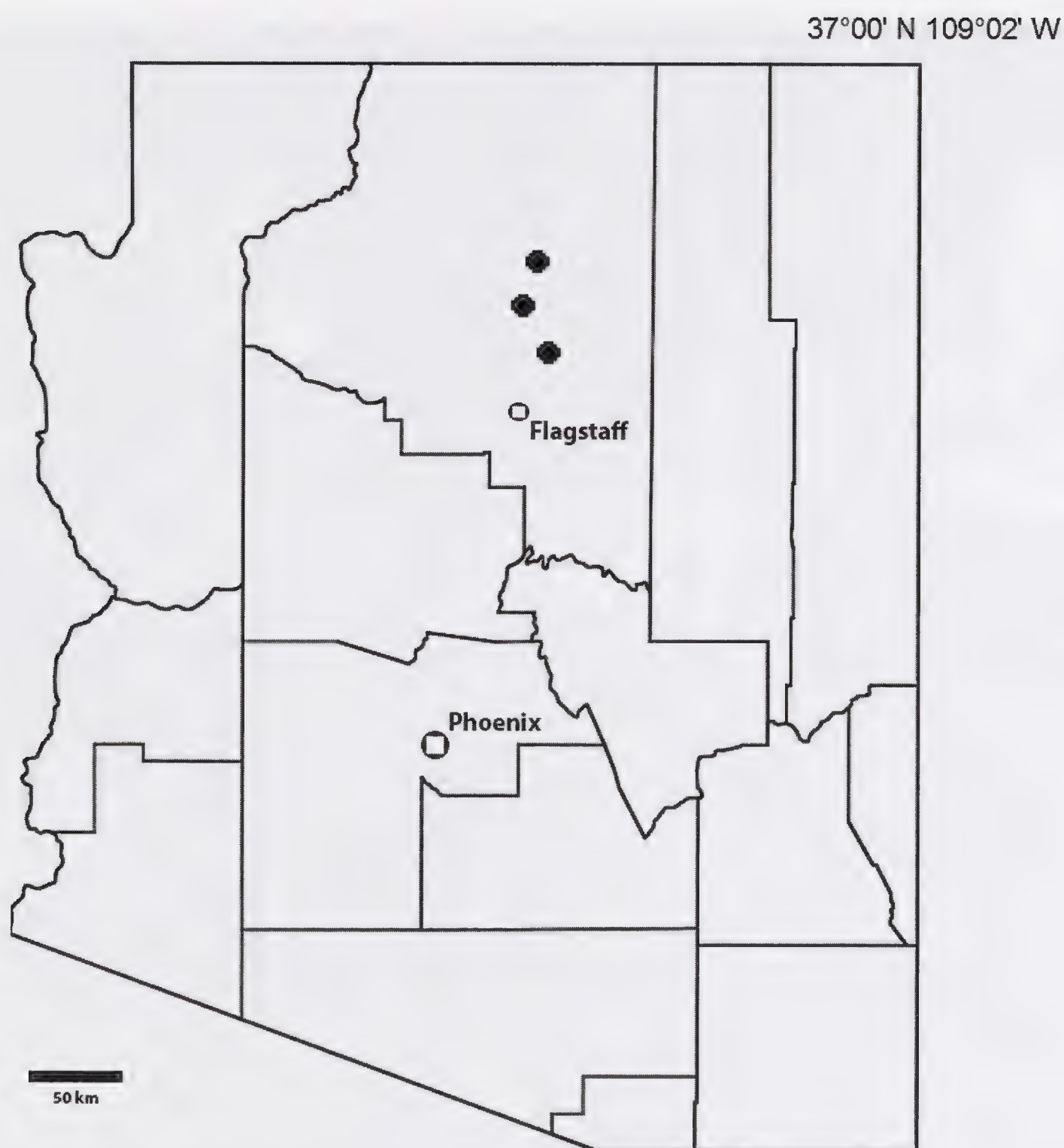


FIG. 4. Distribution map of *Loeseliastrum franciscanum* (Coconino County, AZ). Black circles indicate populations.

development and their lanceolate fruit valves are thick and opaque. *Loeseliastrum schottii* and *L. matthewsii* have divaricately branched stems with eglandular trichomes, long bristles on leaf lobes, and pinnatifid leaves with 5–9(–11) lobes.

PHENOLOGY

Loeseliastrum franciscanum flowers from April to August, and is in fruit from May to September.

DISTRIBUTION AND ECOLOGY

Loeseliastrum franciscanum is geographically closest to *L. schottii* populations at Lake Pleasant in Yavapai Co. (*E. Lehto* L19750, ASU83931) (SEINet) about 180 km SSW of the type locality. The nearest collection of *L. depressum* is 380 km west of Wupatki National Monument in San Bernardino County, CA in the eastern Mojave Desert (*J. André* 19830, UCR243799) (SEINet). Habitat features and population size vary among *L. franciscanum* sites. The most productive site in terms of numbers of individuals is the type locality near Black Bottom Crater between Sunset Crater and Wupatki National Monuments. At Black Bottom Crater, *L. franciscanum* grows in open pinyon-juniper woodlands on gentle slopes in clay soil mixed with cinder pebbles

and larger basalt rocks. In 2013 and 2014, a relatively large number of individuals (>100) were observed growing in ca. 4 hectare area. The species was not detected in adjacent woodlands and scrublands where soils have a thick cinder pebble surface. At CO Bar Ranch, 31 km NW of the type locality, three sites occur less than 3 km apart with fewer than 70 individuals. Here, *L. franciscanum* grows on fine soils derived from eroded Moenkopi Formation in Great Basin Desert Grassland with moderate to heavy disturbance from livestock (Fig. 3). In Hopi Trail Canyon (Navajo Nation), fewer than 50 individuals were detected in fine sediments that accumulated behind large rocks or debris in the wash bottom. A survey of the uplands immediately adjacent to the wash did not yield any additional plants. Consistent features among sites include a narrow elevation range between 1340 and 1780 m, soils consisting of silt or clay, and close proximity to cinder cones.

The limited number of collections, small populations and lack of detection until now indicate that *L. franciscanum* is endemic to the eastern San Francisco volcanic field. Documented locations occur within a 35 km radius of CO Bar Ranch (Fig. 4). The region surrounding Sunset Crater is known for several local endemics including *Phacelia serrata* J. W. Voss, *Eremothera gouldii* (P. H. Raven) W. L. Wagner & Hoch, *Penstemon clutei* A. Nelson, and *Mentzelia*

collomiae Christy (Christy 2008). These species are restricted to the very young soils produced by Sunset Crater and other recent eruptions (Christy 2008). In contrast, *Loeseliastrum franciscanum* occurs on soils that range in age from young quaternary alluvial deposits in Hopi Trail Canyon to the much older Jurassic aged Moenkopi Formation. To better understand the conservation status, degree of endemism, and reasons for endemism, additional surveys

for *L. franciscanum* are needed. These surveys should focus on regions to the east and south of the type locality, specifically Canyon Diablo, Meteor Crater, the Sunset Mountains near Winslow, and the cinder hills around Dilkon. These locations are similar in climate, vegetation, geology, elevation, and topography to known locations. This species is expected to occur within the boundaries of Sunset Crater and Wupatki National Monuments.

KEY TO LOESELIASTRUM SPECIES
Modified from Timbrook (1986)

1. Leaf bristles shorter than leaf lobes; calyx lobe much narrower than intercostal hyaline membrane; capsule valves rotund and translucent
2. Corolla strongly bilabiate, upper lip >2 mm; stamens strongly exerted. *L. franciscanum*
- 2'. Corolla weakly bilabiate, upper lip <2 mm, stamens included. *L. depressum*
- 1'. Leaf bristles equal to or longer than leaf lobes; calyx lobe wider than intercostal hyaline membrane; capsule valves lanceolate and opaque
3. Corolla 11–21 mm, longest filaments equal to or longer than the upper lip *L. matthewsii*
- 3'. Corolla 8–15 mm, longest filaments shorter than the upper lip *L. schottii*

ACKNOWLEDGMENTS

We thank Brittany Burgard for preparing the illustration and Greg Goodwin for photos and collections. Glenn Rink, Mark Porter, Dieter Wilken, and two anonymous reviewers kindly provided helpful comments and insight.

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14859 (ASC); Amargosa Valley about 10 miles west of Lathrop Wells, 22 April 2010, *G. Rink 9121a* (ASC97339); **Mineral Co.:** Terrill Mountains, 7.3 road miles ENE of highway 95 on the more southern road to Rawhide, 8 June 2005, *A. Tiehm 14984* (ASC). UTAH, **Millard Co.:** Deseret, 16 June 1880, *M. E. Jones 1772* (isotype, P00640940 digital image via JSTOR Global Plants).

Loeseliastrum matthewsii: USA, CALIFORNIA, **Inyo Co.:** Camp Independence, 1877, *W. Matthews s.n.* (syntype, GH91178 digital image via JSTOR Global Plants); **Kern Co.:** Northwest of Rosemond, 6 June 2011, *G. Rink 10599* (ASC102645); **Riverside Co.:** Desert flats north of Blythe, east of the McCoy Mountains, 3 March 2011, *G. Rink 10388* (ASC98038); proposed Palo Verde power plant about 15 mi southwest of Blythe, 29 April 2011, *G. Rink 10496* (ASC98132); **San Bernardino Co.:** Mojave Desert, about 35 miles east of Barstow, north of I-40 near the railroad tracks near Pisgah Substation, sandy, 6 May 2010, *G. Rink 9243* (ASC97540); Johnson Valley, about 1 mile south of Galway Dry Lake, 26 March 2014, *G. Rink 12440* (ASC107440).

Loeseliastrum schottii: USA, ARIZONA, **La Paz Co.:** Shea Rd., E of Parker on sand dunes S of road, 28 March 1998, *H. D. Hammond 11352* (ASC64010); **Mojave Co.:** In bottom of Silver Creek Wash, 22 March 1997, *K. Beck s.n.* (ASC62977); 2 miles outside Topoc along old route US 66, 8 April 2001, *J. L. Toney 15* (ASC73125); E of mile marker 7 on White Hills Highway, 1 mi S on Skipper Boulevard, downslope from Prince Albert Mine, 5 April 2008, *D. Peppin 12* (ASC94553); North of Golden Shores and just south of the power plant, east of highway 95, 19 March 2013, *G. Goodwin 4063* (ASC103639); **Yavapai Co.:** Castle Creek Road, 0.1 mi north of intersection of Castle Creek Rd and road to Black Canyon Freeway, just west of Lake Pleasant Regional Park, 31 March 1976, *E. Lehto L19750* (ASU83931 digital image via SEINet). CALIFORNIA, **Inyo Co.:** 2.3 mi N 70 degrees E of Antelope Springs in Deep Springs Valley along Birch Creek, 28 May 1984, *J. D. Morefield 1974c* (ASC); **San Bernardino Co.:** Essex Rest Area, ca. 45 miles W of Needles on I-40, 31 May 1980, *J. R. Kierstead 84* (ASC); Johnson Valley, about 1 mile south of Galway Dry Lake, 26 March 2014, *G. Rink 12439* (ASC).

APPENDIX 1

ADDITIONAL SPECIMENS EXAMINED

Loeseliastrum depressum: USA, CALIFORNIA, **Inyo Co.:** 0.6 mi N 70 degrees E of Deep Springs College, 6 August 1983, *J. D. Morefield 1627a* (ASC). NEVADA, **Nye Co.:** Railroad Valley, 4.1 road miles E of highway 375 on the Nyala road, then 0.7 road miles N, 19 May 2005, *A. Tiehm*

NEVADA, **Clark Co.:** Valley east of Sheep Mountain, east of Jean, 4 April 2009, *G. Rink* 8525 (ASC93732); several miles south of Jean, 8 April 2009, *G. Rink* 8550 (ASC93752); Terrill Mountains, 7.3 road miles ENE of Highway 95 on the more southern road to Rawhide, 8 June 2005, *A. Tiehm* 14985 (ASC). UNKNOWN COUNTRY, Colorado Desert, Sonora, *A. C. V. Schott*, *s.n.* (isotype, GH91181 digital image via JSTOR Global Plants).

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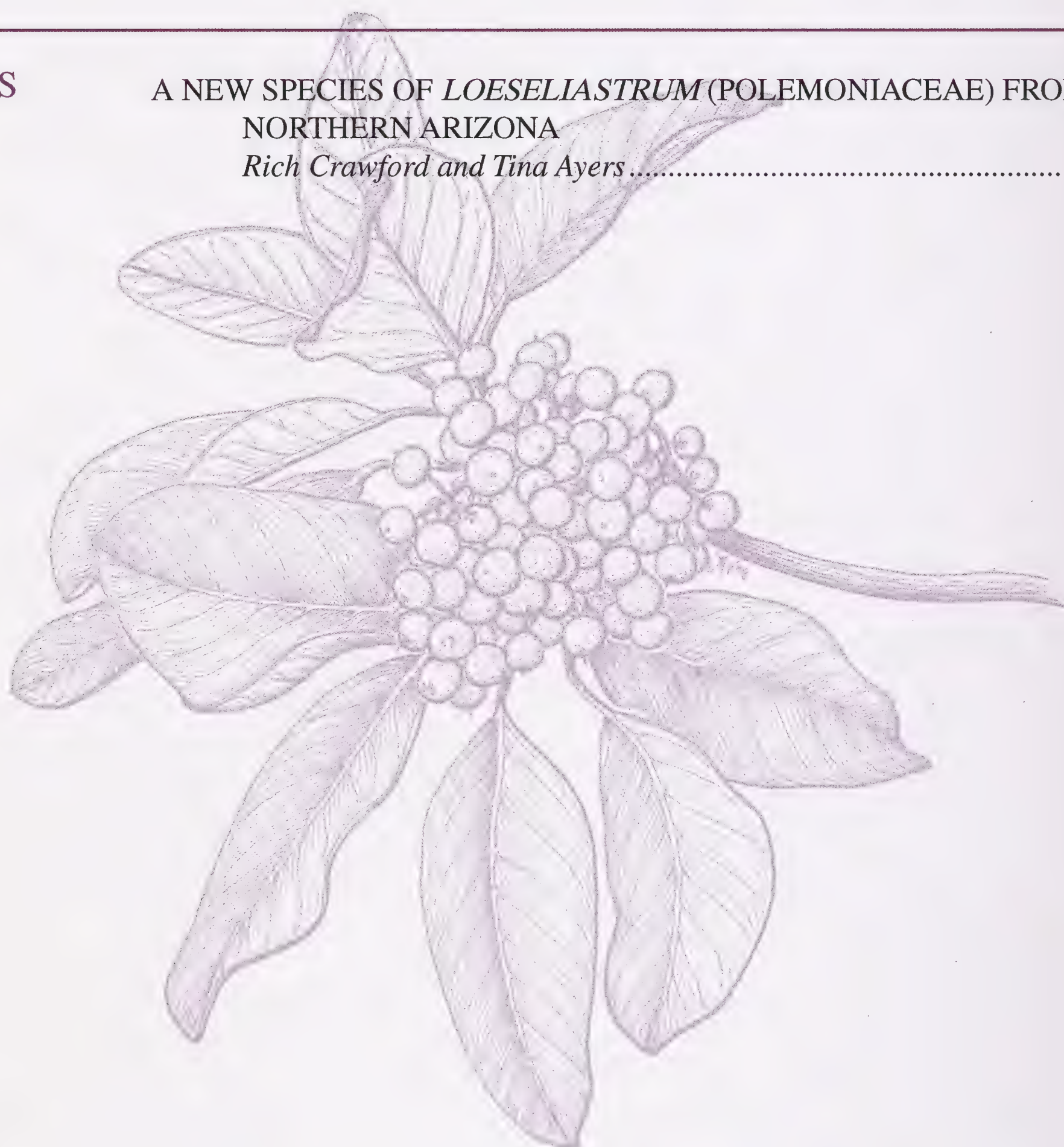
At the time of submission, authors must provide information describing the extent to which data in the manuscript have been used in other papers that are published, in press, submitted, or soon to be submitted elsewhere.



NEW SPECIES

A NEW SPECIES OF *LOESELIASTRUM* (POLEMONIACEAE) FROM
NORTHERN ARIZONA

Rich Crawford and Tina Ayers





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Nemophila menziesii var. *menziesii*, Pepperwood Preserve, CA
Photo: Rachael L. Olliff Yang

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John Powers, University of California, Irvine: Flower scent as a potential reproductive barrier in a Hawaiian plant lineage (Best Overall Presentation)

Mitchell Coleman, California State University, Bakersfield: Seedling recruitment of *Atriplex polycarpa* (Chenopodiaceae) in the San Joaquin Valley of California: The roles of invasive grass competition and residual dry matter (Best Talk)

Maisie Borg, San Francisco State University: Long-term effects of CO₂ enrichment on plant genome and cell size (Joint Winner Best Poster)

Adriana Hernandez, Santa Barbara Botanic Garden: Genotyping using microsatellites shows strong genetic differentiation among populations of the Channel Islands endemic plant *Malva assurgentiflora* (Malvaceae) (Joint Winner Best Poster)



Clockwise from top left: Steve Junak leads 2017 Santa Cruz Island field trip; 26th Graduate Student Symposium at Santa Barbara Botanic Garden; CalBotSoc President Mark Brunell announcing winners of the Symposium; 2017 Annual Banquet in the library of Santa Barbara Botanic Garden.

All photos: Adam Schneider

NOTEWORTHY COLLECTION

ARIZONA

SIDA GLABRA MILL. (MALVACEAE).—Santa Cruz County, Salero Ranch, upper Fresno Canyon (N of Sonoita Creek State Natural Area), 15 km W of Patagonia, 22 km N of the international border, 31.52301, -110.91586 (WGS84), 1182 m, a perennial subshrub, 60 cm tall, flowers yellow, fruits with 5 mericarps, at least 10 plants present, E-facing bouldery margin of seasonal drainage in mesquite grassland, with *Agave schottii* Engelm., *Anisacanthus thurberi* (Torr.) A. Gray, *Anoda abutiloides* A. Gray, *Baccharis sarothroides* A. Gray, *Ericameria laricifolia* (A. Gray) Shinnery, *Fraxinus velutina* Torr., *Gossypium thurberi* Tod., *Penstemon parryi* (A. Gray) A. Gray, *Prosopis velutina* Wootton, *Rhynchosia minima* (L.) DC., *R. texana* Torr. & A. Gray, *Salix gooddingii* C.R. Ball, *Tragia* L. sp., 29 March 2015, Carnahan SC 1070 (ARIZ, DES, USON); Salero Ranch, small drainage leading N from Grosvenor Loop Rd into Grosvenor Hills, 14.5 km W of Patagonia, 23.5 km N of the international border, 31.53521, -110.90648 (WGS84), 1280 m, perennial subshrub, 50 cm tall, stems and leaves hairy, flowers orange-yellow, one plant seen, brushy wash in mesquite grassland, with *Aloysia wrightii* (A. Gray ex Torr.) A. Heller, *Aristida ternipes* Cav., *Artemisia ludoviciana* Nutt., *Baccharis sarothroides*, *Bothriochloa barbinodis* (Lag.) Herter, *Bouteloua curtipendula* (Michx.) Torr., *B. repens* (Kunth) Scribn. & Merr., *Celtis reticulata* Torr., *Cnidoscolus angustidens* Torr., *Disakisperma dubium* (Kunth) P.M. Peterson & N. Snow, *Eysenhardtia orthocarpa* (A. Gray) A. Wats., *Ferocactus wislizeni* (Engelm.) Britton & Rose, *Fouquieria splendens* Engelm., *Gossypium thurberi*, *Mimosa dysocarpa* Benth., *Opuntia engelmannii* Salm-Dyck ex Engelm., *Plumbago scandens* L., *Prosopis velutina*, *Rhynchosia minima*, *Ziziphus obtusifolia* (Hook. ex Torr. & A. Gray) A. Gray, 5 September 2016, Carnahan 1950 (ARIZ). The two collections were 1.7 km apart.

Previous knowledge. *Sida glabra* occurs in Florida, the West Indies, and nearly throughout Mexico, from Sonora and Chihuahua to the Yucatán Peninsula (Fryxell 1988). It also occurs in Central and South America. In Florida it is considered an introduced urban weed (Fryxell and Hill 2015). Native habitat for *Sida glabra* in Mexico includes deciduous forest and savanna (Fryxell 1988); in Sonora it has been documented in desertscrub, tropical deciduous forest, and pine-oak forest, from near sea level to at least 1300 m.

Significance. This is the first report of *Sida glabra* for Arizona and the western United States. The nearest records are 115 km S in a palm canyon SE of Magdalena, Sonora (Whitehead M57 ARIZ; Van Devender s.n. ARIZ). There are additional collections farther south in Sonora in the Sierra del Pinito, the Yécora region, and near Guaymas. The Arizona plants were found on a managed cattle ranch in habitat that is lightly grazed and rarely visited by people; the population is likely a native occurrence.

—SUSAN DAVIS CARNAHAN, Herbarium, University of Arizona, 1130 E South Campus Drive, Tucson, AZ 85721; scarnahan@email.arizona.edu.

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NOTEWORTHY COLLECTIONS

CALIFORNIA

GEMMABRYUM EREMAEUM (Catches. ex J.R. Spence & H.P. Ramsay) J.R. Spence & H.P. Ramsay (BRYACEAE).—Santa Cruz County, on bare soil scattered throughout the Arboretum at the University of California, Santa Cruz (UCSC): 36°59'01.7"N, 122°03'36.4"W, 23 February 2015, *Kellman 7990* (UCSC, CAS); 36°59'02.1"N, 122°03'31.7"W, 26 February 2015, *Kellman 7593* (UCSC); 36°58'53.3"N, 122°03'38.4"W, 9 March 2015, *Kellman 7612* (UCSC).

Previous knowledge. *Gemmabryum eremaeum* is a small bulbiform moss, endemic to arid regions of Australia (Spence and Ramsay 2006).

Significance. This is the first report of *G. eremaeum* for North America. The Arboretum at UCSC has a large collection of Australian plants, and it is possible that diaspores of *G. eremaeum* were inadvertently imported with plant material. Although *G. eremaeum* is found in several places in the Arboretum, it has not yet been seen outside of its boundaries (Kellman 2003). It does not form uniform colonies, but is found scattered amongst other soil mosses, such as *G. gemmilucens* (R. Wilczek & Demaret) J. R. Spence and *Hennediella stanfordensis* (Steere) Blockeel. Therefore, *G. eremaeum* is not expected to be an invasive threat.

Bulbils in the leaf axils are a key character in identifying species in *Gemmabryum* sect. *Gemmabryum* (Spence 2014). Bulbils of Australian specimens of *G. eremaeum* have an apical groove, with two small leaf primordia at each end of the groove. The California specimens have the same apical groove, but lack the leaf primordia. All California specimens were shown to J. R. Spence for determination, and he felt the lack of the leaf primordia was not significant

for identification (Spence, personal communication). California specimens also share the hyaline awn at the leaf apex with Australian material, the character that immediately separates this taxon from any other bulbil-bearing *Gemmabryum* in North America (Spence 2014), and the character that attracted me to it in the field. I attempted to borrow specimens from Australian herbaria for comparison, but none were available for international loan.

—KENNETH M. KELLMAN, Bryophyte Curator, William T. Doyle Bryophyte Herbarium, Kenneth S. Norris Center for Natural History, University of California, Santa Cruz, CA 95064; kkellman@ucsc.edu.

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LINKING SEEDLING SPATIAL PATTERNS TO SEED DISPERSAL PROCESSES IN AN INTERMITTENT STREAM

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ABSTRACT

To better understand the colonization of bare riparian sites, I examined seedling distribution patterns and seed dispersal processes of 11 common riparian species. The study site was two in-channel sedimentation basins on an intermittent stream flowing into the Tijuana River Valley, southern California. The 11 species relied on one of two vectors to disperse seeds into the basins, either water or wind. Seven species dispersed to the basins via winter stream flows and, of these, three grew mainly at the basin periphery and four grew mainly in the bed. Seed buoyancy tests and tests of dispersal using seed mimics showed that, for these species, the main process producing seedling distributions was that of sorting of the seeds by the stream flows based on seed buoyancy. Most past work on seed dispersal in riparian habitats has been conducted in perennial systems where non-buoyant seeds usually remain in deep water and never germinate, and this has led to the notion that non-buoyant seeds are not effectively dispersed by water. This study shows that non-buoyant seeds are effectively dispersed by flows in intermittent systems because the seeds were able to germinate and grow once the bed had dried. Four species dispersed to the basins via wind, and their seedlings grew in concentric bands around the basins. Monitoring of seed production and tracking of the basin's water level showed that, for these species, seedling distributions were the result of staggered periods of seed dispersal and gradual decline of the water level. Only three of these 11 species were abundant in the five- and 22-year old stands examined, i.e., the wind-dispersed *Baccharis salicifolia* (Ruiz & Pav.) Pers., *Salix lasiolepis* Benth., and *S. gooddingii* C.R. Ball. A banding pattern established at the time of recruitment, with *S. lasiolepis* higher on the bank than *S. gooddingii*, was retained in the older forests, making this one of very few examples that show a direct link between seed dispersal processes, seedling distributions, and adult distributions. This study provides insight into the early development of riparian woodlands by identifying and explaining distribution patterns in water- and wind-dispersed colonizers.

Key Words: anemochory, hydrochory, natural restoration, pleustochory, seed buoyancy.

Within river corridors, bare areas of riverbank can be quickly colonized by a suite of riparian plants that arrive from elsewhere. Rapid colonization is a common phenomenon, yet the seed dispersal processes that underlie seedling establishment are not well understood (Briggs 1996; Gurnell et al. 2006). Because the spatial distribution of the colonizers may persist for decades as a forest matures, understanding colonization is of enormous value (Willson and Traveset 2000; Boland 2014a).

Riparian plants rely on two main vectors to disperse their seeds and other propagules—flowing water and wind. Dispersal via flowing water, termed hydrochory, has been shown to be important in the transport of riparian plant seeds (Tabacchi et al. 2005) and establishment of riparian species in disturbed areas (Gurnell et al. 2006, 2008). Seed buoyancy is considered essential for water-dispersal, as most of the seeds entering riparian corridors are deposited along bankfull drift lines with other buoyant debris, particularly after seasonal floods (Vogt et al. 2006, 2007). Emphasis in the study of water-dispersal has been on long-distance dispersal along rivers (Johansson et al. 1996), but the influence that water-dispersed seeds have on riparian vegetation community composition and structure is still

being debated (Danvind and Nilsson 1997; Andersson et al. 2000; Levine and Murrel 2003; Jansson et al. 2005; Nilsson et al. 2010).

Seed dispersal via wind, termed anemochory, is typical of many riparian trees, such as willows (*Salix* L. spp.) and cottonwoods (*Populus* L. spp.). Seeds of wind-dispersed plants are usually small and have hairs or outgrowths that increase their surface area to catch the wind (Matlack 1987; Karrenberg et al. 2002). The seed shadow of wind-dispersed plants typically peaks near the parent plant and declines rapidly with distance outwards (Willson 1983; Bullock and Clarke 2000), and most studies of wind-dispersal, like those of water-dispersal, have focused on long-distance dispersal (Nathan and Muller-Landau 2000; Nathan et al. 2002). Unlike water-dispersal, wind-dispersal has been shown to be a key factor in structuring some riparian woodlands in southern California (Boland 2014a).

Here I examine the colonization of large bare areas to better understand the processes that produce spatial patterns in a young riparian community. The bare areas were within two sedimentation basins that were cleaned out each fall and so allowed observation of a new crop of recruited seedlings each year. Initial observations showed that some colonizing

species arrived via water-dispersal during winter flows and others arrived via wind-dispersal during spring. My general approach in this study was to describe the patterns in the distribution of recruiting seedlings and to link these patterns to the underlying processes that produced them. In particular, I addressed the following questions:

1. Among the water-dispersed species, what is the distribution of seedlings within the basins, and can the distributions be attributed to the buoyancy characteristics of their seeds?
2. Among the wind-dispersed species, what is the distribution of seedlings within the basins, and can the distributions be attributed to the timing of their seed dispersal?
3. What roles do the colonizing species play in the structure of older riparian woodlands and forests?

This study of the distribution of riparian seedlings is one of very few that examines water-dispersal and wind-dispersal simultaneously and is the first to do so in an intermittent stream.

METHODS

Study Site

This research was conducted from 2012–2015 at the Goat Canyon sedimentation basins in Border Field State Park, Tijuana River Valley, San Diego County, California (32°32.459'N, 117°6.374'W). The two earthen, in-channel basins constituted a 520 m reach of an intermittent stream that drains a small, coastal watershed (<20 km²). The basins, which together hold approximately 45,000 m³ of sediment, were cleaned out each year in fall (September and October). The clean-out removed all plants, seeds, and debris, along with the accumulated sediment. River flows then refilled the basins with water and sediment during the rainy season (November to March). Deposited sediment was composed of mostly fine sand and silt (Boland 2014b). After the rainy season, the basins contained large, shallow pools (13,000 to 15,000 m²) that slowly drained and evaporated during spring and summer and eventually dried completely by the end of summer (Fig. 1). Apart from the sediment removal in fall, the basins were left undisturbed and seedlings were allowed to establish naturally and, thus, the basins provided a suitable site at which to examine seed dispersal and patterns of seedling recruitment.

Plant Species

This research focused on 11 plant species that recruited abundantly in the basins and in disturbed riparian sites elsewhere in the valley. These included species that were water- and wind-dispersed, native and non-native, annual and perennial, and forbs, shrubs and trees (Table 1). For ten of these, the dispersing propagule was a single seed, and for one (*Xanthium strumarium* L.) it was a bur containing

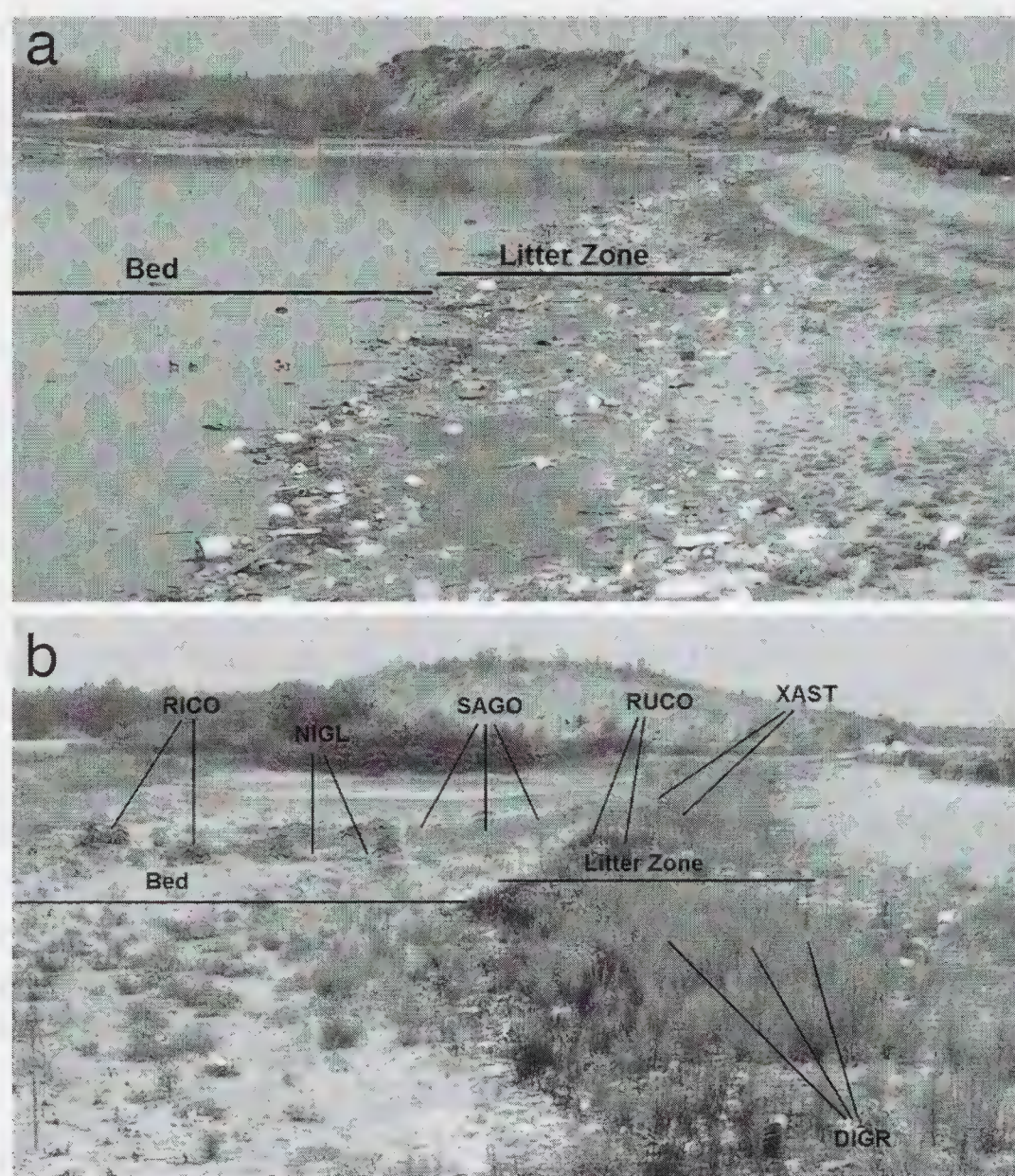


FIG. 1. The lower Goat Canyon sedimentation basin during March (above) and September (below). In March, the water level is a little below the bankfull maximum; the Litter Zone is exposed and the Bed is underwater. In September, the pool has dried exposing both the Litter Zone and the Bed. The Litter Zone is densely vegetated with several species, including the water-dispersed *Rumex conglomeratus* (RUCO) and *Xanthium strumarium* (XAST) and the Bed is sparsely vegetated with several species, including the water-dispersed *Ricinus communis* (RICO) and *Nicotiana glauca* (NIGL). The wind-dispersed species are present in bands; two are indicated—*Dittrichia graveolens* (DIGR) and *Salix gooddingii* (SAGO).

two seeds; for convenience, the term ‘seed’ was used in this paper for all 11 species.

Seedling Pattern #1—Water-Dispersed Species

a) *Seedling distribution.* To document the seedling distribution pattern of the seven water-dispersed species, the basin area that was underwater during the winter floods was divided into two zones: the Litter Zone and the Bed (Fig. 1). The Litter Zone, so named because it contained abundant buoyant trash, was the high zone around the basin periphery near bankfull stage. In summer 2014, this zone was 6–29 m wide and had a total area of 8,446 m². The Bed was the rest of the basin down-slope from the Litter Zone. It contained little or no buoyant trash. In summer 2014, the Bed was 36–60 m wide and had a total area of 19,963 m². Fifteen stratified-random transects (20 m long) were placed in each zone during August and September 2014. Along each transect, seedlings of the water-dispersed species were counted within ten quadrats (75 × 75 cm) placed at two-meter intervals, for a total of 150 quadrats per zone (i.e., 10

TABLE 1. Characteristics of the common species in the Goat Canyon sedimentation basins. Origin is native (N) or non-native (X); growth form is forb (F), shrub (S) or tree (T); life span is annual (A) or perennial (P); seed length measurement is for the seed alone and, where applicable, for the seed plus the length of the seed’s attached hairs or pappus (given in parentheses). The cocklebur seed length is for the bur. All details are from Jepson Manual (Baldwin 2012), except ‘a’ which are from Lightner (2011) and ‘b’ which are from Boland (unpublished; n = 10–20 dispersing seeds).

Species	Dispersal method	Common name	Origin	Growth	Life span	Seed length (mm)
<i>Rumex conglomeratus</i> Murray	Water	Whorled dock ^a	X	F	P	1.5–2.0
<i>Xanthium strumarium</i> L.	Water	Cocklebur	N	F	A	10–30+
<i>Persicaria lapathifolia</i> (L.)	Water	Willow weed	N	F	A	1.5–3.2
<i>Ricinus communis</i> L.	Water	Castor bean	X	S	P	9–22
<i>Nicotiana glauca</i> Graham	Water	Tree tobacco	X	S	P	0.5–0.7 ^b
<i>Solanum americanum</i> Mill.	Water	White nightshade ^a	N	F	P	1.0–1.5
<i>Datura wrightii</i> Regel	Water	Jimson weed	N	F	P	5.0
<i>Dittrichia graveolens</i> (L.)	Wind	Stinkwort	X	F	A	2.0 (3–5)
<i>Salix lasiolepis</i> Benth.	Wind	Arroyo willow	N	T	P	0.8–1.2 (10–15) ^b
<i>Baccharis salicifolia</i> (Ruiz & Pav.) Pers.	Wind	Mule fat	N	S	P	0.8–1.3 (3–6)
<i>Salix gooddingii</i> C.R. Ball	Wind	Goodding’s black willow	N	T	P	0.8–1.2 (10–15) ^b

quads × 15 transects). For each species, abundance in the two zones was tested against the expected values of 50% in each zone using the G-test for goodness-of-fit (Sokal and Rohlf 1995).

I developed a simple index called the Litter Zone Percentage to describe the overall distribution of each species. The index indicated a species’ relative abundance in the Litter Zone and was calculated in the following manner:

$$\text{Litter Zone Percentage}_X = (D_L \cdot A_L)(100)/(D_L \cdot A_L + (D_B \cdot A_B))$$

where D_L is the average density of species x in the Litter Zone, A_L is the total area of the Litter Zone, D_B is the average density of species x in the Bed, and A_B is the total area of the Bed. A species with a Litter Zone Percentage of more than 50% had most of its seedlings in the Litter Zone, whereas a species with a Litter Zone Percentage of less than 50% had most of its seedlings in the Bed.

b) *Seed buoyancy.* To measure the floating ability of seeds of the water-dispersed species, ripe seeds were collected in the Goat Canyon area in November 2014, prior to the first winter rains. The seeds were stored dry at room temperature, and their buoyancy was tested three times in the lab during the following December and January. Each trial started on the first day of a rainstorm when the seeds likely would have been dispersed by stream flows. In each trial, 50 seeds of each species were placed in empty seven-oz plastic cups, ten seeds per cup, except for *X. strumarium*, which had one bur per cup. Approximately 125 ml of water was then poured into each cup and the number of floating seeds was counted at the following times: 0.1, 0.5, 1, 2.5, 4, 6.5, 12, 24, 36, 48, and 72 hr after initial immersion. Before each count, the seeds in each cup were sprayed with water from a mister to dislodge any that were floating because of surface tension alone. Other researchers have stirred seeds for the

same reason (e.g., Danvind and Nilsson 1997) but I found that stirred seeds adhered to the stirrer and sides of the cup so spraying was more effective. Seed buoyancy was calculated for each species as the average length of time seeds floated, with a maximum of 72 hr. The strength of the link between seed buoyancy and seedling distribution (Litter Zone Percentage) was quantified using correlation analysis (Sokal and Rohlf 1995).

c) *Dispersal of seed mimics.* To test whether buoyant seeds accumulated in the Litter Zone and non-buoyant seeds accumulated in the Bed, I released buoyant and non-buoyant seed mimics upstream of the basins. Buoyant seed mimics were ping-pong balls (Momentum Brands by Dollar Empire LLC, Vernon, CA; recreational grade; 40 mm diameter; weight = 2.7 g) and non-buoyant seed mimics were ping-pong balls filled with sand (mean weight = 42.4 ± 3.8 g). Non-buoyant balls were prepared by drilling a hole in the ball, inserting the end of a streamer, filling the ball with sand, and sealing the hole with hot glue. The streamer, added to aid in the detection of buried balls, was made of polypropylene curling ribbon (0.5 cm wide and 75 cm long). Just before a rainstorm, I placed fifty balls of each type together in the dry streambed, approximately 50 m upstream of the basins. This was done on two occasions: 22 April 2015 and 8 May 2015. The balls were washed downstream with the subsequent storm flows and over the next few weeks, as the pools dried, I searched for the balls. The location of each ball was mapped using a handheld GPS unit (Garmin eTrex Venture HC) and determined to be in the Litter Zone or the Bed of the basin, based on the positions of the zone of stranded buoyant trash and the thalweg. Abundances of the buoyant and non-buoyant seed mimics in the two zones were compared using the G-test of independence (Sokal and Rohlf 1995).

Seedling Pattern #2—Wind-Dispersed Species

a) Seedling distribution and water level. To describe the seedling distribution pattern of the four wind-dispersed species, I used methods similar to those used in a prior study (Boland 2014b) and suited to the high seedling densities and narrowly banded distributions. I first monitored the decline of the water's edge and then surveyed the seedlings along that timeline. During winter 2014, a vertical transect was started at a randomly-chosen site around the lower basin, and the water's edge was marked with wooden stakes every 3–4 wk from 13 December 2014 to 20 July 2015. The stakes established a timeline of the lowering water level and the associated moist fringe of sediment around the pool. In August 2015, a vertical transect (15 m) was placed next to the timeline transect, from the highest to the lowest water levels, and seedlings of the wind-dispersed species were counted within quadrats (20×20 cm) placed end-to-end along the transect for a total of 75 quadrats.

b) Timing of seed production by adults. Seed production of the four wind-dispersing species was monitored to determine their periods of seed dispersal. In *Dittrichia graveolens*, the percent cover of fruiting flower heads was recorded every 3–12 d for the same 15 adult plants in Goat Canyon from 11 December 2014 to 12 January 2015. In the other three species, adult female plants were monitored weekly from 5 February 2013 through 25 June 2013. Each week the same individuals of *Salix lasiolepis* ($n = 12$), *Salix gooddingii* ($n = 15$), and *Baccharis salicifolia* ($n = 12$) were visited in the Tijuana River Valley, and the percent cover of fruiting catkins (or flower heads in the case of *B. salicifolia*) was estimated following the procedure described in Boland (2014a). A mean percent cover was calculated for each species on each monitoring date. All percent cover estimates were of mature, fluffy seeds that were ready to disperse. The strength of the link between the species' seedling distributions and timing of seed dispersal was quantified using correlation analysis (Sokal and Rohlf 1995).

Community Development

To describe the early successional changes that occur after recruitment and the roles played by the 11 species in developing riparian woodlands, I conducted surveys at three Tijuana River Valley sites with woodland stands of different ages. Surveys were conducted in summer 2015, and the stand ages were 0.5 yr (Goat Canyon lower basin), 5 yr (Site B at Dirt Road), and 22 yr (Site 1 at Bisect). The latter two are young forests described in Boland (2014a). At each site, the riparian habitat was divided into three strata based on elevation—High (floodplain), Intermediate (bankfull stage), and Low (bed)—and a 25-m transect was randomly placed in

each stratum perpendicular to the slope. Along each transect, the percent cover of each of the 11 species was determined using the line-intercept method, and an additional 250 m² was searched to mark as present or absent any of the 11 species not found along the transect. Also, along each transect at the 0, 12.5, and 25 m positions, maximum canopy height was measured with a meter stick, stiff meter tape, or laser distance measurer (Bosch DLR130K) and percent canopy cover measurements were taken 1.15 m above ground level using a spherical densiometer (Forest Densiometers).

RESULTS

Seedling Pattern #1—Water-Dispersed Species

a) Seedling distribution. The water-dispersed species had many seedlings in the basins during summer, with densities up to 7.91 seedlings per m² (Table 2). Seedlings of all seven species were significantly more abundant in one or the other of the two zones (G-test for goodness-of-fit; $P < .01$). *Rumex conglomeratus* Murray, *X. strumarium* and *Persicaria lapathifolia* (L.) Gray were significantly more abundant in the Litter Zone and had Litter Zone Percentages of 85% or greater. In contrast, *Ricinus communis* L., *Nicotiana glauca* Graham, *Solanum americanum* Mill., and *Datura wrightii* Regel were significantly more abundant in the Bed and had Litter Zone Percentages of 10% or less.

b) Seed buoyancy. The seven species fell into two groups: *R. conglomeratus*, *X. strumarium*, and *P. lapathifolia* had buoyant seeds with average floating times of 23.5 hours or longer; whereas *R. communis*, *N. glauca*, *S. americanum*, and *D. wrightii* had sinking seeds with average floating times of 5.1 hr or less (Table 2). Seedling distribution (Litter Zone Percentage) was significantly correlated with seed buoyancy ($r^2 = 0.755$; $n = 7$; $P < 0.05$) indicating that species with buoyant seeds grew mainly in the Litter Zone, whereas species with sinking seeds grew mainly in the Bed.

c) Dispersal of seed mimics. Ninety-five of the 100 buoyant balls released were recovered, and 96 of the 100 non-buoyant balls released were recovered, a recovery rate of 95% and 96%, respectively. The buoyant and non-buoyant balls accumulated in different areas of the basins. Most of the buoyant balls (93 of 95, or 98%) were found in the Litter Zone and all of the non-buoyant balls (96 of 96, or 100%) were found in the stream thalweg and Bed. The distributions of buoyant and non-buoyant balls were significantly different (G-test of independence: $G = 245$, $P < .001$), indicating that differences in buoyancy could account for their dispersal into different zones. These results strongly suggest that seedling distributions of the seven water-dispersed

TABLE 2. Seedling densities and seed buoyancies of water-dispersed species. Seedling densities are the average number of individuals per m² in the two zones—Litter and Bed (n = 150 quadrats per zone). Seed buoyancy is the average number of hours afloat (n = three trials; 72 hr maximum). The G statistic is for the g-test for goodness-of-fit (an * indicates the test was done using the Yates continuity correction); Std d. = standard deviation; n = number of seedlings counted.

Species	Density					n	G statistic	P	Litter zone percentage	Buoyancy	
	Litter zone		Bed zone		Avg					Std d.	
	Avg	Std d.	Avg	Std d.							
<i>Rumex conglomeratus</i>	7.91	11.32	0.00	0.00	667	924	<0.001	100%	71.6	0.6	
<i>Xanthium strumarium</i>	4.05	6.47	0.21	0.77	360	356	<0.001	89%	23.5	4.9	
<i>Persicaria lapathifolia</i>	0.46	1.64	0.04	0.25	42	36.6	<0.001	85%	34.3	7.4	
<i>Ricinus communis</i>	0.28	1.11	1.03	2.40	111	37.9	<0.001	10%	5.1	0.3	
<i>Nicotiana glauca</i>	0.19	1.01	1.16	2.14	114	65.5	<0.001	6%	4.3	2.3	
<i>Solanum americanum</i>	0.01	0.15	0.84	1.66	72	89.2	<0.001	1%	0.1	0.0	
<i>Datura wrightii</i>	0.00	0.00	0.12	0.57	10	9.9*	<0.01	0%	3.9	1.1	
Total	12.91		3.40								

species can be attributed to the buoyancy characteristics of their seeds.

Seedling Pattern #2—Wind-Dispersed Species

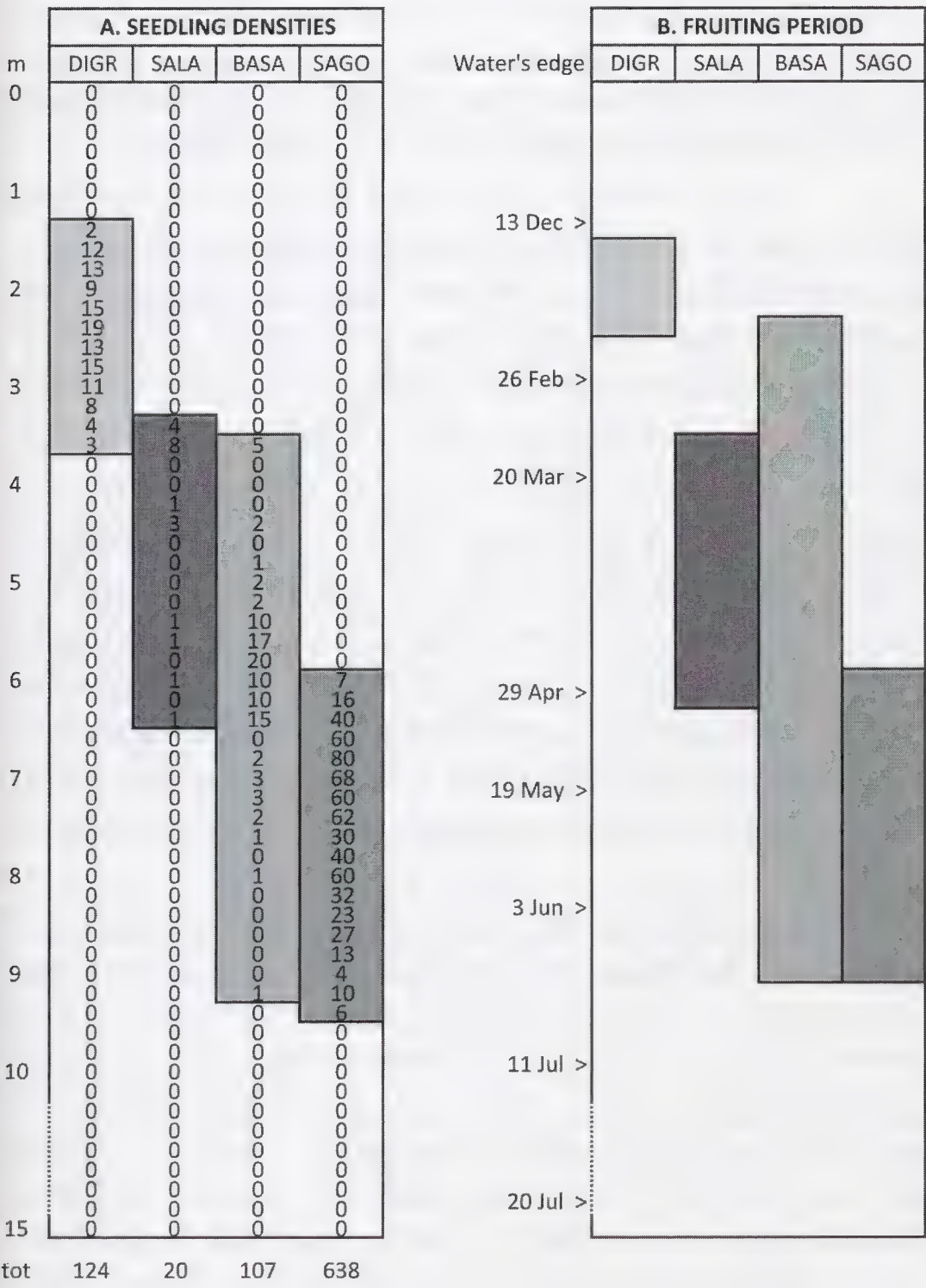


FIG. 2. Seedling densities and fruiting periods of the wind-dispersed species along the vertical transect. Seedling densities are the number of seedlings per quadrat (400 cm²); the distribution of each species is enclosed in a shaded box. Fruiting periods are from Fig. 3, displayed on the vertical transect according to the position of the water's edge on given dates; the fruiting period of each species is enclosed in a shaded box. DIGR = *D. graveolens*, SALA = *S. lasiolepis*, BASA = *B. salicifolia* and SAGO = *S. gooddingii*.

a) *Seedling distribution and water level.* The four wind-dispersed species had abundant seedlings in the basins during summer with densities up to 80 seedlings per quadrat (Fig. 2A), or 2,000 seedlings per square meter. Seedlings of three of the species were distributed at relatively distinct levels along the transect and grew in concentric bands around the basin; *Dittrichia graveolens* (L.) Greuter formed the highest band, *S. lasiolepis* the middle band, and *S. gooddingii* the lowest band. *Baccharis salicifolia* seedlings were distributed more widely and overlapped with those of *S. lasiolepis* and *S. gooddingii*. The water level in the basin was highest on 13 December 2014 and declined gradually during spring and summer 2015 until August, when the entire transect line site was dry (Fig. 2). The moist fringe at the water's edge, so important for recruitment, moved gradually down the transect with the declining water level.

b) *Timing of seed production by adults.* The four wind-dispersed species had different periods of seed production (Fig. 3). The seeds of three species were ripe and ready for dispersal during relatively short, discrete periods: *D. graveolens* dispersed first with a peak in December, *S. lasiolepis* dispersed next with a peak in early April, and *S. gooddingii* dispersed later with a peak in late May. *Baccharis salicifolia* dispersed seeds over a longer period that overlapped *S. lasiolepis* and *S. gooddingii*. When the timing of seed production was displayed on the timeline for the position of the water's edge (Fig. 2B), the seed production pattern largely matched the seedling distribution pattern of the four species (Fig. 2A). In both seedling densities and fruiting period, *D. graveolens* formed the highest band, *S. lasiolepis* the middle band, *S. gooddingii* the lowest band and *B. salicifolia* was widely overlapping. Along this time-

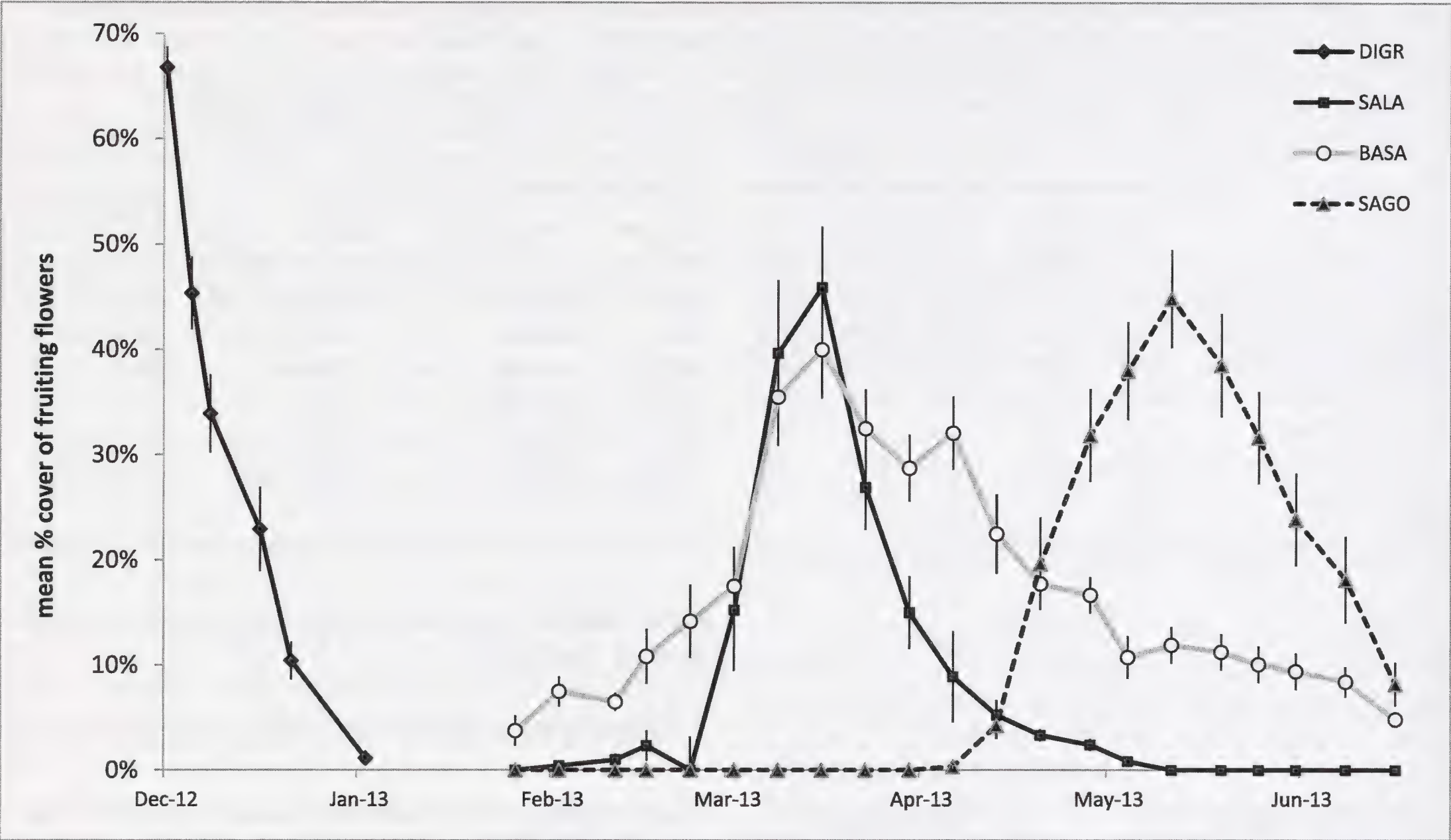


FIG. 3. Fruiting periods of the four wind-dispersed species. Data are mean percent cover (± 1 std. err.) of fruiting flowers on adult plants. DIGR = *D. graveolens*, BASA = *B. salicifolia*, SALA = *S. lasiolepis* and SAGO = *S. gooddingii*.

line, the densities of the seedlings were significantly correlated with the mean percent cover of dispersing seeds ($r = 0.638$; $P < 0.01$, $n = 140$). These results strongly suggest that seedling distributions of the four wind-dispersed species (i.e., the banding pattern) can be attributed to the timing of their seed dispersal and the simultaneous decline of the water level.

Community Development

Of the 11 species common as seedlings in the basins, only three were abundant in the older stands (Table 3). These species were *S. lasiolepis*, *S. good-*

dingii, and *B. salicifolia*, and they formed the main structural components of the older woodlands and forests in the Tijuana River Valley. *Baccharis salicifolia* dominated the moderately tall (3–5 m) woodlands surrounding the tall (8–17 m), dense riparian forests dominated by *S. lasiolepis* and *S. gooddingii*. The *S. lasiolepis*-higher and *S. gooddingii*-lower pattern, first seen in the seedlings (Fig. 2A), was still apparent in the 5 and 22 yr old forests; *S. lasiolepis* was the dominant species in the Intermediate stratum and *S. gooddingii* was the dominant species in the Low stratum in both the 5 and 22 yr old forests. Most of the other species that were abundant

TABLE 3. Percent cover values for the 11 species in riparian stands of increasing age. Data are for the High, Intermediate (Int.), and Low strata within each stand. P = present, canopy height is the maximum for the transect ($n = 3$), and canopy cover is the average for the transect ($n = 3$).

Age of stand	0.5 yr			5 yr			22 yr		
Zone	High	Int.	Low	High	Int.	Low	High	Int.	Low
Canopy height (m)	1.2	0.6	0.4	3.2	9.3	8.2	4.3	10.5	17
Canopy cover (%)	15	0	0	91	99	99	6	98	99
<i>Rumex conglomeratus</i>	69%	P							
<i>Xanthium strumarium</i>	70%		3%						
<i>Persicaria lapathifolia</i>	10%								
<i>Ricinus communis</i>		6%	16%	P	3%	44%	P	8%	P
<i>Nicotiana glauca</i>		1%	4%						
<i>Solanum americanum</i>			4%	P					
<i>Datura wrightii</i>		1%	13%						
<i>Dittrichia graveolens</i>	2%	2%							
<i>Salix lasiolepis</i>	P	1%	P	24%	100%	76%		96%	
<i>Baccharis salicifolia</i>	2%	P	P	90%	P		50%	20%	
<i>Salix gooddingii</i>		65%	P		50%	85%		12%	96%

in the basins were not present in the older woodlands, for instance *R. conglomeratus*, *X. strumarium*, *P. lapathifolia*, *N. glauca*, *Datura wrightii*, and *Dittrichia graveolens* were absent from the older woodlands.

DISCUSSION

Two Seedling Patterns and the Processes that Produced Them

Understanding patterns and the processes that produce them is a central theme in ecology (e.g., Turner et al. 2010). Here I have described two superimposed seedling distribution patterns, one formed by water-dispersed species and the other by wind-dispersed species, and I have identified the processes that produced them.

The seedling distribution pattern of water-dispersed species was straightforward; species with buoyant seeds grew high on the bank around the basin periphery, and species with sinking seeds grew in the bed. As the release of seed mimics demonstrated, river flows could sort seeds according to their buoyancy, and deposit them in different parts of the basin. Sorting of seeds based upon their buoyancy was therefore the main process that produced the seedling distribution pattern of these species.

Sorting of seeds according to their buoyancy has been shown in experimental flumes (Merritt and Wohl 2002; Chambert and James 2009), and floating seeds are thought to disperse like the 'flotation load,' while sinking seeds are carried in the water column with other non-buoyant material such as bed-load sediment and non-buoyant trash (Goodson et al. 2003; Gordon et al. 2004; Markwith and Leigh 2008; Chambert and James 2009). But sorting of seeds according to their buoyancy has rarely been examined in the field because most research on seed dispersal has been done in perennial streams, where seeds that sink typically remain in deep water and never germinate. Sinking seeds have therefore come to be considered ineffectively dispersed by flowing water (Andersson et al. 2000) or simply lost to the system (e.g., Murray 1986; Brown and Chenoweth 2008). While this may well be the case in perennial streams, this study showed that sinking seeds were effectively dispersed by flows in an intermittent system, as they germinated when the bed became exposed. Hence, non-buoyant seeds should not be overlooked in intermittent streams; the dispersal of non-buoyant seeds may be common in intermittent streams and should receive more attention in areas such as southern California, where an estimated 49% to 73% of streams are intermittent (Mazor et al. 2012).

The seedling distribution pattern of wind-dispersed species was quite different to that of water-dispersed species; wind-dispersed species grew in concentric bands on the basin's banks, each at a level on the bank that reflected the timing of their

seed production and the decline of the water level. With the gradual decline in water level during spring and summer, the moist fringe of sediment suitable for seed germination gradually moved lower on the bank, such that seeds from later-dispersing species recruited lower on the bank. The staggered timing of seed dispersal and the gradual change in the position of moist fringe were therefore the main processes that produced the seedling distribution of the wind-dispersed species. This seedling pattern was apparent because the gradually lowering pool surrounded by open sediment was ideal for seedlings to develop (Mahoney and Rood 1998). This banding pattern was also seen at three disturbed sites along the Tijuana River during 2010 (Boland 2014a). It is interesting to note that Van Splunder et al. (1995), working on the River Waal in the Netherlands, documented the sequential seed production of four Salicaceae species but found no clear patterns in the seedling distributions because water levels rose and fell several times during the seed production period, effectively mixing together the seedling distributions of the early- and later-dispersing species.

In addition to being blown through air, some wind-dispersed seeds have been shown to have a secondary mode of dispersal, which involves being pushed by the wind, or 'sailing,' on the surface of the water, termed pleustochory (Boland 2014b). Willow seeds, for example, land on a pool's surface then sail, via pleustochory, directly to their recruitment safe sites at the pool's edge. In essence, a pool can act as a collector of wind-dispersed seeds, and pleustochory serves to concentrate and deposit those seeds in their safe sites at the pool's edge. The concentration of seeds in the narrow band of moist sediment ideal for germination results in seedling densities that are orders of magnitude greater than that expected from the seed rain alone (Boland 2014b). In the current study, pleustochory concentrated and deposited both wind- and buoyant water-dispersed seeds at the pool periphery and, because it also acted on floatable debris, it helped to create the Litter Zone around the pool (Boland, personal observations).

Researchers who study dispersal typically lump buoyant water-dispersed seeds with 'buoyant' wind-dispersed seeds (Hyslop and Trowsdale 2012). It is clear, however, that the two are associated with distinctly different life histories. The water-dispersed species in this study, for example, (1) produced seeds well in advance of the winter rainy season, (2) produced seeds that could remain dormant for long periods, (3) dispersed during winter, (4) were distributed according to buoyancy characteristics of their seeds, and (5) were generally short-lived members of the community. The wind-dispersed species (1) produced seeds mainly after the rainy season, (2) produced seeds that had no dormancy and, instead, are known to have 'Very Fast Germination' (Parsons 2012; Boland 2014b; Boland unpublished data), (3) dispersed mainly during spring and summer, (4) were distributed according to seed

production period and water level decline, and (5) were generally long-lived members of the community, as three of the four species grew to become the dominants in older riparian forests. Thus, buoyant water-dispersed seeds and 'buoyant' wind-dispersed seeds represent different strategies and are best considered separately. Furthermore, it is instructive to understand that when seedlings of two species are growing together in an area, they may have arrived at different times and via different dispersal pathways.

Useful Simplifications

The Goat Canyon sediment basins were not completely natural stream reaches, but they had features that were useful for the study of seed dispersal and early community development. They were cleaned out in fall, naturally refilled with fresh sediment in winter, and left undisturbed long enough for the colonizing plant community to develop. They had no vegetation or seed bank, and had earthen bed and banks with a full range of floodwater elevations within which normal river processes were able to act. Together, these features were useful when teasing apart the superimposed seedling distribution patterns and related dispersal processes.

Ping-pong balls, as used in this study, were simple and effective seed mimics. They are normally lightweight and buoyant, but could be made to be non-buoyant and yet retain a uniform size and shape. Other researchers have used colored seeds in experimental flumes (Merritt and Wohl 2002; Chambert and James 2009) and floating wooden cubes along rivers (Nilsson et al. 1991; Andersson et al. 2000) to mimic water-dispersed seeds, and now ping pong balls can be added to the list of useful and acceptable seed mimics.

Community Development

Of the colonizing species in this study, three wind-dispersed species (*B. salicifolia*, *S. lasiolepis* and *S. gooddingii*) persist and become the dominant structural elements in early successional riparian forests in the Tijuana River Valley. Seedlings of these species quickly grow into dense thickets and then into tall, even-aged, dense stands. The banding pattern established at the time recruitment, with *S. lasiolepis*-higher and *S. gooddingii*-lower, is retained for decades (Table 3) and has been previously documented in three other clearings in the natural river (Boland 2014a). Therefore, differences in the timing of dispersal of these willows lead to both the banding pattern of their seedlings and zonation of their adults. This is one of very few examples showing a direct link between seed dispersal and the distribution of adult trees in natural habitats (Schupp and Fuentes 1995; Traveset et al. 2014). Other examples involve dispersal of seeds by monkeys (Russo 2005) and birds (Wenny 2000; Jordano and Schupp 2000).

On the other hand, the water-dispersed colonizing species do not appear to play a major role in structuring riparian communities in the Tijuana River Valley. These species tend to be herbaceous short-lived, weedy species that colonize disturbed sites and raise the local diversity but disappear from older stands as, presumably, they are outcompeted by taller-growing, longer-lived species. If water-dispersed species are the short-lived, weedy species in other riparian systems, it would explain why researchers have thus far found it so difficult to show that water-dispersal influences the long-term composition and structure of riparian communities (Andersson et al. 2000; Levine and Murrell 2003; Jansson et al. 2005).

Restoration Project Applications

Some riparian restoration sites are prepared with initial grading that removes most vegetation, topsoil and seed bank, and such sites, at least in southern California, may be somewhat similar to the Goat Canyon sedimentation basins. The results presented here show that a graded site can be colonized naturally by desirable native species and, if left undisturbed, can develop into a riparian forest with natural density, diversity and dispersion (Boland 2014a). Making use of the riparian habitat's considerable capacity for natural recovery is called 'natural restoration' or 'passive revegetation' (Faber et al. 1989; Briggs 1996; Sher et al. 2002; Boland 2014a). To be most successful, a natural restoration project should aim to maximize recruitment of the wind-dispersed species that become the dominant riparian forest trees, and to do so by ensuring that the water's edge sweeps across the restoration site during the seed dispersal period of those species (March to June in southern California). This type of natural restoration is likely to be particularly effective at sites that are cleared beforehand, have abundant seed sources nearby, include a pool with a declining water level, and are left undisturbed after the seedlings colonize (Briggs 1996). The results of this study suggest that knowing the mode and timing of seed dispersal will allow for a more accurate prediction of natural recruitment and will therefore be extremely helpful in the natural revegetation of restored riparian areas.

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GENETIC DIVERSITY AND POPULATION STRUCTURE IN THE RARE, ENDEMIC BAKER CYPRESS (*HESPEROCYPARIS BAKERI*)

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ABSTRACT

Baker cypress (*Hesperocyparis bakeri* [Jeps.] Bartel) is one of 10 species of cypress found in western North America. It is restricted to a small number of highly disjunct, isolated populations, making it particularly vulnerable to the influences of genetic drift, inbreeding, and reduced gene flow. Baker cypress is fire adapted and the serotinous cones require heat to open and release seeds. Altered fire regimes have negatively impacted the health and vigor of some populations and lower levels of genetic diversity could make this species more susceptible to the impacts of predicted future climate change. Previously, no information on genetic diversity and population structure of Baker cypress was available. We used 12 polymorphic allozyme loci to assess genetic diversity and population structure for eight of the 11 known populations of Baker cypress. Overall mean observed heterozygosity (H_o) was 0.178 and expected heterozygosity (H_e) was 0.204, values higher than for other cypress species and other fire adapted conifers. Although genetic diversity was relatively high, many populations had a deficiency of heterozygotes (fixation index > 0), most likely due to inbreeding and possibly a Wahlund effect. Population differentiation among seven of the eight populations (northernmost population excluded) was 9%, considerably lower than for other conifers with disjunct populations. Our results indicate that the current population structure of the species is likely a fairly recent reduction from a formerly widespread distribution with differentiation among populations resulting from genetic drift. Implications of genetic diversity and population structure for potential restoration work are discussed.

Key Words: Baker cypress, endemism, fire adaptation, genetic diversity, *Hesperocyparis bakeri*, population structure, restoration, serotiny.

Small, isolated populations within a species often have lower levels of gene flow, reducing the exchange of genetic material. Gene flow can help a species adapt to changes in their environment, but the introduction of new alleles can also swamp alleles in the target population that are locally adapted (Frankham et al. 2002). Populations of long-lived plants such as forest trees have to be able to adapt to their local environment to survive both annual variations in temperature and rainfall as well as long-term changes in climate. In order to track climatic changes during glacial and interglacial periods during the Quaternary period (roughly 1.8 million yr bp to present), tree species have experienced large-scale range shifts, population contractions and expansions, and aggregation and disassociation of plant communities (Davis and Shaw 2001, Petit et al. 2008). More recently, land-use changes have dramatically altered the landscape, especially in mixed-conifer forests, increasing both the density and dominance of shade tolerant species (Merschel et al. 2014). These shade-tolerant species have benefited from these changes, but this often comes at the expense to other species, especially fire dependent species such as Oregon white oak (*Quercus garryana* Douglas ex Hook.) (Devine and Harrington 2006), whitebark pine (*Pinus albicaulis* Engelm.) (Arno and Hoff 1989), and Baker cypress (*Hesper-*

ocyparis bakeri [Jeps.] Bartel) (Merriam and Rentz 2010). In addition, climate change has altered temperature and rainfall patterns (IPCC 2014) and this is occurring at a pace that exceeds the abilities of many forest tree species to migrate in response to these changes (Aitken et al. 2008).

There are ten *Hesperocyparis* species native to California, eight of which are endemic (Baldwin et al. 2012) and all are comprised of relatively small, disjunct and isolated populations. The majority of these species have little or no published information on genetic diversity (heterozygosity) and population structure. Species with small, shrinking, or isolated populations may be genetically vulnerable because they often are prone to the influences of genetic drift, genetic bottlenecks, and higher levels of inbreeding than more widespread species with continuous population distributions. It should also be noted that there has been taxonomic instability of *Cupressus* L. in the last decade (Terry 2012), with the introduction of the genus *Xanthocyparis* by Farjon (2002) and the movement of western hemisphere cypresses (originally *Cupressus*) first to the genus *Callotropsis* (Little 2006), then to *Hesperocyparis* (Adams et al. 2009). *Hesperocyparis* is currently used by the Jepson eFlora (Jepson Flora Project 2017) and the USDA Plants database (USDA NRCS 2017) for

TABLE 1. Site names and characteristics for eight Baker cypress populations sampled for genetic analysis (from Merriam and Rentz 2010).

Site no.	Site name	Latitude	Longitude	Est. size (ha)	Elevation (m)	Avg. tree age (range)	Soil type
1	Goosenest	41.7396	−122.2583	120	1500–1850	98 (53–163)	Volcanic
2	Flounce Rock	42.7343	−122.6036	1.2	1230	132 (108–174)	Metasedimentary
3	Miller Lake	42.0677	−123.2957	20	1230–1600	N/A	Metasedimentary
4	Timbered Crater	41.2271	−121.4481	2800	1070–1230	70 (25–125)	Volcanic
5	Wheeler Peak	40.1032	−120.7034	30	1970–2120	95 (46–158)	Volcanic
6	Burney	40.7713	−121.6097	600	1385–1540	56 (2–96)	Volcanic
7	Seiad	41.9145	−123.1487	320	925–1170	68 (32–113)	Ultramafic
8	Hamburg	41.7646	−123.1063	6.4	1350–1600	149 (116–196)	Ultramafic

North American cypresses and is what we have used here.

Baker cypress (*Hesperocyparis bakeri*) is a rare endemic currently found in eleven widely scattered and isolated populations in northern California and southwestern Oregon. In the Siskiyou Mountains it occurs on serpentine and granitic soils, while in the Cascades and Sierra Nevada it occurs on volcanic soils (Esser 1994). Baker cypress is unique in that its range includes the northernmost (Flounce Rock) (Callahan 2013), as well as the highest elevation (Wheeler Peak) population of cypress in North America (Keeler-Wolf 1985); the Wheeler Peak population is the furthest population of cypress from the coast in California (Keeler-Wolf 1985).

Baker cypress is relatively long lived and is wind pollinated; life history traits often associated with high genetic diversity and population connectivity, and lower interpopulation differentiation (Hamrick and Godt 1989, Hamrick et al. 1992, Hamrick and Godt 1996). However, it also is an early seral, endemic species with a very limited distribution, serotinous cones, and gravity-dispersed seed, life history traits that would be consistent with lower genetic diversity, higher inbreeding and significant interpopulation genetic subdivision (Loveless and Hamrick 1984). Due to the small size of many of the extant populations, and the cumulative impacts of fire exclusion, there is a need for restoration in some Baker cypress populations. However, while Baker cypress has been included in phylogenetic analyses of western hemisphere cypresses (Bartel et al. 2003, Little 2006, Mao et al. 2010, Terry et al. 2012), no information on genetic diversity and population structure of the species has been available to date, which is important for any restoration treatments to ensure that, at the minimum, genetic diversity is not lost.

Population geographic range sizes in the eleven known stands of Baker cypress range from <1.2 to over 2800 hectares; eight of these populations are less than 140 hectares, with three populations being less than 8 hectares (Merriam and Rentz 2010) (Table 1). Some of the smallest populations are in the poorest condition and in need of restoration (Merriam and Rentz 2010). Merriam and Rentz (2010) documented stand health by assessing crown condition, stand age

structure and density, fuel loading, cones per tree and viable seeds per cone, and presence of regeneration.

Baker cypress is fire adapted and stand replacing fires generally produce conditions favorable to regeneration by removing duff and heating the cones so that seeds are released. In several populations, fire exclusion is leading to the replacement of Baker cypress by shade tolerant species, senescence, and generally poor stand conditions. One of these populations that was in very poor condition, Mud Lake (not sampled in this study), burned in the 2007 Moonlight Fire and substantial post-fire regeneration of Baker cypress has been observed (Merriam and Rentz 2010).

The objective of this study was to assess genetic diversity and population differentiation across the range of the species to determine 1) the level of genetic diversity present within and among populations, and 2) if geographically isolated populations are genetically distinct to aid in the development of management plans for restoration or population conservation of this species.

METHODS AND MATERIALS

Tissue Collection

Branch tips with current-year’s foliage were collected from approximately 30 individuals per population for eight of the 11 populations that comprise the known distribution of Baker cypress (Fig. 1, Table 1). Samples from most sites were collected in June 2012 with the exception of the Seiad stand, which was collected in September 2012. Individual tree samples were placed in sealed plastic bags and stored in a cooler with ice packs or refrigerator until delivered to the USDA Forest Service National Forest Genetics Laboratory (NFGEL) in Placerville, CA for processing and genetic marker analysis. Samples were delivered to NFGEL within 6 d of collection.

Allozyme Analysis

Allozyme markers were utilized for this study, as they are a simple, inexpensive, and efficient method for assessing genetic diversity and population structure. In addition, the numerous studies of conifers

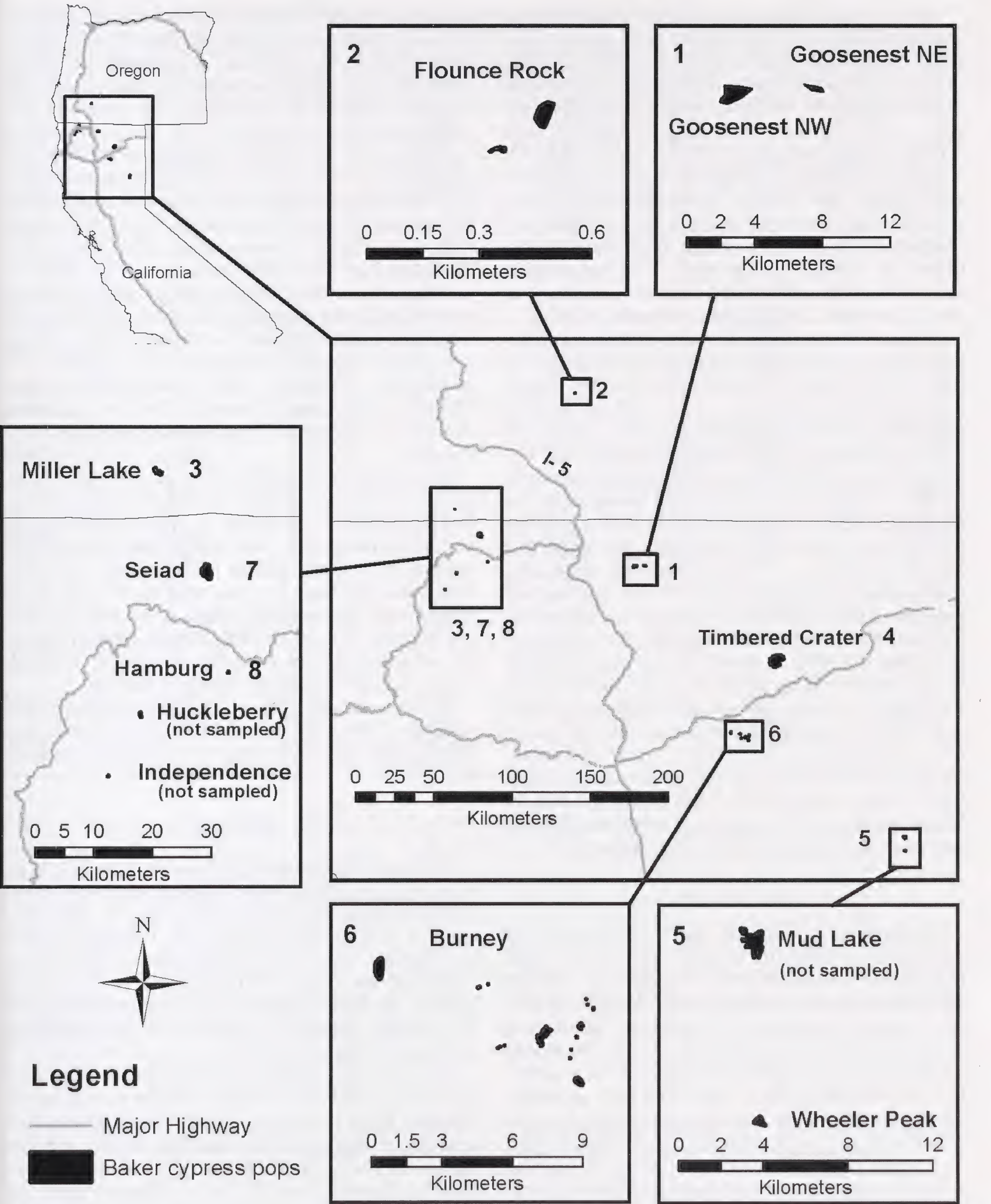


FIG. 1. Species distribution of Baker cypress, including unsampled populations. Numbers refer to populations in Table 1.

using allozymes (Conkle 1987, Hamrick and Godt 1996, Truesdale and McClenaghan 1998, Ledig et al. 2005) allows for a direct comparison with other related species or species with similar distributions or ecological strategies. At the time of this work, DNA based markers were not available for analysis of this species; allozymes, while maybe less informative than some markers available now, were used as they would yield sufficient data to address the questions of interest.

Approximately 100 mg of leaf tissue per tree was ground to fine powder in mortars with pestles under liquid nitrogen. A total of 0.5 ml of a 0.1 M Tris-HCl (pH 8.0) extraction buffer, with 10% (w/v) polyvinylpyrrolidone-40, 10% sucrose, 0.17% EDTA (Na₂ salt), 0.15% dithiothreitol, 0.02% ascorbic acid, 0.10% bovine albumin, 0.05% NAD, 0.035% NADP, and 0.005% pyridoxal-5-phosphate (Pitel and Cheliak 1984, USDA Forest Service 2012) was added to the powder to create a slurry. Slurry was transferred to microtiter plates and samples were frozen at -70°C . On the day of electrophoresis, samples were thawed and the extracts were absorbed onto 3 mm wide chromatography paper wicks.

Methods of sample preparation and electrophoresis follow the general methodology of Conkle et al. (1982), with some modifications (USDA Forest Service 2012). All enzymes were resolved on 11% starch gels. A lithium borate electrode buffer (pH 8.3) was used with a Tris citrate gel buffer (pH 8.3) (Conkle et al. 1982) to resolve phosphoglucumutase (PGM), malic enzyme (ME), and phosphoglucose isomerase (PGI). A sodium borate electrode buffer (pH 8.0) was used with a Tris citrate gel buffer (pH 8.8) (Conkle et al. 1982) to resolve glutamate-oxaloacetate transaminase (GOT), triose-phosphate isomerase (TPI), glucose-6-phosphate dehydrogenase (G6PD), and uridine diphosphoglucose pyrophosphorylase (UGPP). A morpholine citrate electrode and gel buffer (pH 6.1) (USDA Forest Service 2012) was used to resolve phosphogluconate dehydrogenase (6PGD), malate dehydrogenase (MDH), isocitrate dehydrogenase (IDH), and shikimate dehydrogenase (SKD). Enzyme stain recipes follow USDA Forest Service (2012). For quality control, all individuals were run and scored twice. In addition, gels were photographed for future reference.

Data Analysis

Genetic diversity of the eight populations was assessed using GenAlEx (Peakall and Smouse 2006, Peakall and Smouse 2012) to calculate the population mean numbers of alleles, observed and expected heterozygosity, and the fixation index (inbreeding coefficient, measured as F) and their associated standard errors. GenAlEx calculates $F = 1 - (H_o/H_e)$, and Wright's (1965) F -statistics were estimated per allele using mean heterozygosities across populations as $F_{is} = 1 - (H_o/H_e)/H_e$; $F_{it} = 1 - (H_o/H_t)$; $F_{st} = 1 - (H_e/H_t)$, where H_t is the total expected heterozygosity. Genetic differentiation among populations was assessed from pairwise F_{st} values and an analysis of molecular variance (AMOVA) (Excoffier et al. 1992) also using GenAlEx. A Bayesian model-based analysis was used to infer population genetic structure using STRUCTURE version 2.3.3 (Pritchard et al. 2000). Data were analyzed using a no admixture ancestry model as these are fully discrete populations and this model is often more powerful at detecting subtle structure (Pritchard 2010). In this

model, correlated allele frequencies were used to estimate the posterior probabilities $L(K)$ of K groups and the individual percentages of group membership assigned according to their multilocus profiles (Falush et al. 2007). We assessed the probabilities for a range of K ($K = 1 - 8$) using a burn-in period of 10,000 iterations of the Markov chain Monte Carlo model, followed by a run length of 500,000 iterations with the analysis of each level of K repeated 10 times. We calculated Delta K up to $K = 8$ according to Evanno et al. (2005) and used Structure Harvester (Earl and von Holdt 2012) to visualize the results. The correlation between mean population linear geographic distance and both mean linearized genetic distance and linearized F_{st} among populations was assessed using a Mantel (1967) test in GenAlEx. GenAlEx uses a modification of the Haversine Formula (Sinnott 1984) to calculate distances in kilometers from latitude/longitude coordinates.

The program BOTTLENECK (Cornuet and Luikart 1997) was used to test whether populations had undergone a restriction in effective population size in the recent past. The infinite alleles model was used as it is recommended for allozyme data. The Wilcoxon sign-rank test was used as it is the most appropriate and powerful when fewer than 20 loci are available (Piry et al. 1999). The minimum number of polymorphic loci available was five at Flounce Rock, and the maximum was 11 at Burney.

The correlation of H_o , H_e , F with average tree age and stand size, as reported by Merriam and Rentz (2010) was calculated using SAS software, version 9.3.

RESULTS

Of the 14 loci examined, 12 were polymorphic in at least one population (Table 2). PGI1 and GOT2 were monomorphic, while GOT1 had just one heterozygote in each of the Burney and Timbered Crater populations. There were only two private alleles, with a rare private allele for TPI with just two heterozygotes in the Burney population, and a private allele at a moderate frequency for MDH1 in the Hamburg population (Table 2).

Overall mean observed heterozygosity (H_o) was 0.178 and population mean H_o ranged from 0.100 at Flounce Rock to 0.224 at the Hamburg population, and mean expected heterozygosity (H_e) was 0.204 (range 0.104–0.260) (Table 3). The percent polymorphic loci varied from 35.7% at Flounce Rock to 78.6% at the Burney population, and effective number of alleles varied from 1.36 at Flounce Rock to 2.14 at Burney (Table 3). For all populations except Wheeler Peak, expected heterozygosity exceeded observed heterozygosity resulting in positive values of F (mean F was 0.099) (Table 3). The mean Wright's F_{is} (0.070) was similar to the value of F (Table 4). The value of F for Wheeler Peak was only very slightly negative (-0.043) but not significantly

TABLE 2. Allele frequencies for 14 allozyme loci from eight Baker cypress populations.

Locus	Allele	Goosenest	Flounce Rock	Miller Lake	Timbered Crater	Wheeler	Burney	Seiad	Hamburg
ME	1	0.783	0.883	0.406	0.85	0.833	0.667	0.593	0.726
	2	0.15	0.117	0.5	0.133	0.117	0.333	0.37	0.274
	3	0.067	—	0.094	0.017	0.05	—	0.037	—
PGM	1	0.667	1	0.643	0.65	0.783	0.5	0.648	0.694
	2	0.233	—	0.214	0.2	0.217	0.232	0.278	0.145
	3	0.1	—	0.143	0.15	—	0.268	0.074	0.161
PGI1	1	1	1	1	1	1	1	1	1
PGI2	1	0.517	1	0.771	0.767	0.683	0.533	0.722	0.516
	2	0.483	—	0.229	0.133	0.317	0.433	0.222	0.484
	3	—	—	—	0.1	—	—	0.037	—
	4	—	—	—	—	—	0.033	0.019	—
TPI	1	1	1	1	1	1	0.967	1	1
	2	—	—	—	—	—	0.033	—	—
GOT1	1	1	1	1	0.983	1	0.983	1	1
	2	—	—	—	0.017	—	0.017	—	—
GOT2	1	1	1	1	1	1	1	1	1
G6PD	1	0.55	0.75	0.5	0.65	0.8	0.733	0.556	0.887
	2	0.45	0.25	0.5	0.35	0.2	0.267	0.444	0.113
UGPP	1	0.967	1	1	1	0.933	0.867	0.907	0.919
	2	—	—	—	—	0.017	0.033	—	—
	3	0.033	—	—	—	0.033	0.083	0.074	0.081
	4	—	—	—	—	0.017	0.017	0.019	—
6PGD	1	0.683	1	0.171	0.367	0.117	0.533	0.13	0.548
	2	0.317	—	0.829	0.467	0.883	0.467	0.722	0.419
	3	—	—	—	0.167	—	—	0.148	0.032
SKD1	1	0.233	0.483	0.257	0.117	0.2	0.15	0.148	0.484
	2	0.633	0.517	0.429	0.567	0.8	0.75	0.741	0.419
	3	0.133	—	0.314	0.317	—	0.1	0.111	0.097
MDH1	1	1	1	1	1	1	1	1	0.613
	2	—	—	—	—	—	—	—	0.387
MDH2	1	0.783	0.933	0.914	1	1	0.817	1	0.774
	2	0.217	0.067	0.086	—	—	0.183	—	0.226
IDH	1	1	0.15	0.971	1	1	0.967	1	1
	2	—	0.85	0.029	—	—	0.033	—	—

different from zero ($SE = 0.040$). Genetic diversity in the Flounce Rock population was lower for all statistics, with the exception of fixation index (F). Excluding the Flounce Rock population mean values were: $H_o = 0.189$, $H_e = 0.218$, $F = 0.106$.

The AMOVA showed that 18% of the observed genetic variation resided among populations while the remaining 82% was among individuals within populations. Overall Wright's F_{st} was 0.171 ($SE = 0.058$) (Table 4), but excluding Flounce Rock the overall F_{st} was reduced by nearly one half ($F_{st} =$

0.089. $SE = 0.027$). The common and rare alleles for the IDH locus in the Flounce Rock population are opposite to the other populations (Table 2), leading to very large values of F_{it} and F_{st} (Table 4). To assess the impact of this locus on overall F_{st} , the analysis was repeated excluding the IDH locus. With IDH excluded overall F_{st} decreased to 0.117 ($SE = 0.031$), which is similar to the value for all loci excluding the Flounce Rock population indicating that this locus contributed significantly to the genetic differentiation of Flounce Rock from the remaining populations.

TABLE 3. Sample size and mean effective number of alleles (N_e), percent polymorphic loci (%P), observed heterzygosity (H_o), expected heterozygosity (H_e), and fixation index (F) for 14 allozyme loci. Standard errors in parentheses.

Site	N	A (SE)	%P	H_o (SE)	H_e (SE)	F
Goosenest	30	1.79 (0.21)	57.10%	0.190 (0.055)	0.229 (0.063)	0.143
Flounce Rock	30	1.36 (0.13)	35.70%	0.100 (0.042)	0.104 (0.045)	0.02
Miller Lake	35	1.79 (0.21)	57.10%	0.175 (0.053)	0.221 (0.067)	0.136
Timbered Crater	30	1.86 (0.25)	50.00%	0.186 (0.064)	0.202 (0.067)	0.091
Wheeler Peak	30	1.71 (0.24)	50.00%	0.150 (0.046)	0.145 (0.044)	−0.043
Burney	30	2.14 (0.23)	78.60%	0.222 (0.054)	0.257 (0.060)	0.11
Seiad	27	2.00 (0.30)	50.00%	0.180 (0.055)	0.211 (0.062)	0.159
Hamburg	31	1.86 (0.21)	64.30%	0.224 (0.059)	0.260 (0.062)	0.131
Grand Mean		1.81 (0.08)	55.40%	0.178 (0.019)	0.204 (0.021)	0.099

TABLE 4. Estimates of Wright's (1965) F-statistics for 12 polymorphic loci in Baker cypress.

Locus	F _{is}	F _{it}	F _{st}
ME	0.267	0.339	0.098
PGM	0.209	0.263	0.068
PGI2	0.057	0.168	0.118
TPI	−0.034	−0.004	0.029
GOT1	−0.017	−0.004	0.013
G6PD	0.03	0.103	0.075
UGPP	0.28	0.306	0.037
6pgd	0.062	0.345	0.303
SKD1	0.177	0.253	0.093
MDH1	−0.088	0.299	0.356
MDH2	0.032	0.125	0.096
IDH	−0.13	0.738	0.768
Mean	0.070 (0.036)	0.244 (0.053)	0.171 (0.058)

Pairwise F_{st} values among populations ranged from 0.025 to 0.251 (Table 5), although excluding the Flounce Rock population, the highest value was only 0.091 between the Hamburg and Seiad populations. The mean pairwise F_{st} value between Flounce Rock and the other seven populations was 0.172, while the mean among all the other populations was 0.051. The second highest pairwise F_{st} value (0.222) was between Flounce Rock (the northernmost population) and Wheeler Peak (the southernmost population). Unexpectedly, the greatest pairwise F_{st} value was between Flounce Rock and Miller Lake, the geographically closest population to Flounce Rock (Fig. 1). The three Siskiyou populations (Miller Lake, Seiad, and Hamburg) are geographically close and while the pairwise F_{st} value between Miller Lake and Seiad was one of the smallest among all pairwise comparisons (0.025), the values between Hamburg and Seiad (0.091) and Miller Lake (0.089) were among the largest (Table 5). When the IDH locus was excluded, pairwise F_{st} values between Flounce Rock and all other populations were still higher, often by an order of magnitude, than among the remaining populations.

Analysis of genetic structure using the programs STRUCTURE and Structure Harvester revealed five population groups (greatest mean of estimated $\ln(K)$ and Delta K at $K = 5$) (Figs. 2, 3a and b). The STRUCTURE plot (Fig. 2) clearly shows that population 2 (Flounce Rock) was differentiated from all other populations and comprised one of the five groups, as was also clear from the pairwise F_{st}

values (Table 4). Figure 2 also shows that population 8 (Hamburg) comprised another group. The remaining populations: 1, 3, 4, 5, 6 and 7 (Goosenest, Miller Lake, Timbered Crater, Wheeler Peak, Seiad, and Burney) comprised the three remaining groups with approximately equal contributions of all three groups in all of these populations. Interestingly, Miller Lake, Seiad, and Hamburg are geographically closest, yet Hamburg did not cluster genetically with these populations. The correlation of both genetic distance and F_{st} with geographic distance tested with the Mantel test did not show a significant relationship between genetic and geographic distance indicating that there does not appear to be isolation by distance among the populations sampled.

The BOTTLENECK analysis indicates that there is evidence of recent reductions in effective population size at half of the populations sampled (Table 6). This Wilcoxon sign-rank test gives the probability of observing expected heterozygosity under the null hypothesis (mutation-drift equilibrium) that is different from the probability under the alternative hypothesis (a bottleneck). An unexpected result is that there does not seem to be a relationship of this test statistic with population geographic size. The significance of the test statistic for Flounce Rock, the smallest population, approached significance ($P = 0.089$), while Timbered Crater, the largest population was marginally significant ($P = 0.055$). The populations with the strongest evidence of having experienced a bottleneck were Hamburg (6.4 hectares), Miller Lake (20 hectares), and Goosenest (120 hectares).

Genetic diversity statistics (H_o , H_e , F) were not related to latitude or longitude (data not shown). All three variables showed a negative relationship with average tree age and a positive relationship with population geographic size, however, the correlations were not significant.

DISCUSSION

Genetic diversity in almost all populations of Baker cypress included in the study was relatively high, with some evidence of past bottlenecks and inbreeding. In addition, with the exception of the small Flounce Rock population, there was moderate to low levels of population differentiation.

TABLE 5. Pairwise population F_{st} values.

	Goosenest	Flounce Rock	Miller Lake	Timbered Crater	Wheeler Peak	Burney	Seiad
Flounce Rock	0.174	-					
Miller Lake	0.062	0.251	-				
Timbered Crater	0.039	0.165	0.043	-			
Wheeler Peak	0.076	0.222	0.055	0.042	-		
Burney	0.018	0.159	0.044	0.037	0.046	-	
Seiad	0.059	0.215	0.025	0.03	0.031	0.034	-
Hamburg	0.053	0.187	0.086	0.073	0.089	0.039	0.091

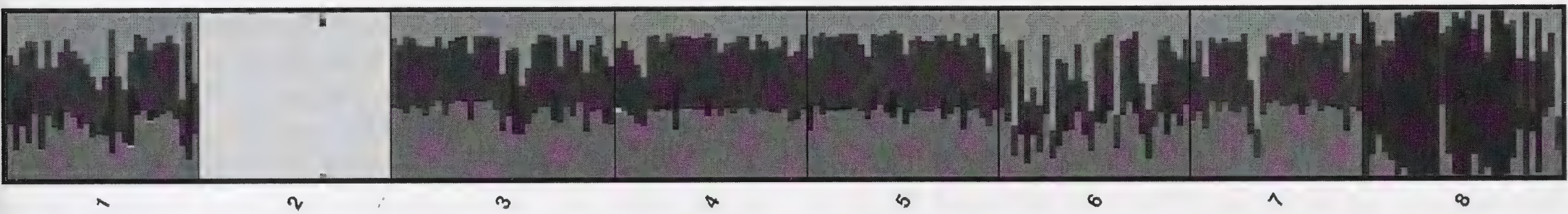


FIG. 2. STRUCTURE bar chart showing population composition. Numbers (x-axis) refer to populations in Table 1.

Genetic Diversity

The expected heterozygosity (H_e) value of Baker cypress (mean 0.204, SE 0.021), even when the anomalously low heterozygosity Flounce Rock population was included, was higher than reported values for two other cypress species, Tecate cypress (*Hesperocyparis forbesii* Jeps.) (mean 0.112, SE 0.019) (Truesdale and McClenaghan 1998), Monte-

rey cypress (*H. macrocarpa* Hartw. ex Gordon) (mean 0.177, SE 0.035) (Conkle 1987), as well as Brewer spruce (*Picea breweriana* S. Watson) (mean 0.129, no SE given) (Ledig et al. 2005), another relictual species endemic to the Klamath region (Wolfe 1964). H_e for Baker cypress was also higher than knobcone, Bishop, and Monterey pines (*Pinus attenuata* Lemmon, *P. muricata* D. Don, and *P. radiata* D. Don), three species of fire-adapted, closed-

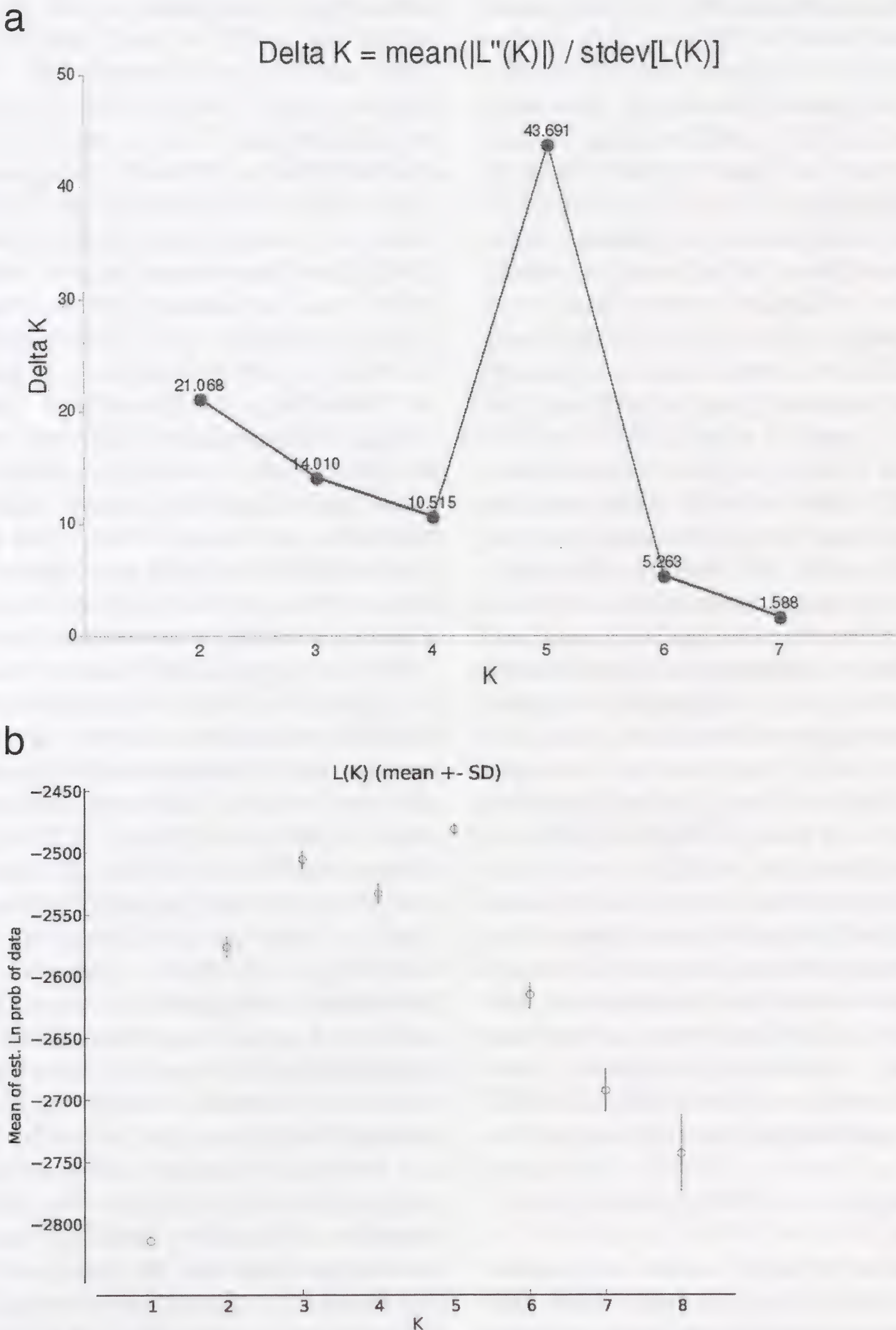


FIG. 3. A) Delta K and B) mean ln (K) from Evanno et al (2005) showing K = 5 indicating 5 distinct genetic groups.

TABLE 6. Wilcoxon sign-rank test for recent bottlenecks in eight Baker cypress populations under the infinite alleles model of mutation-drift equilibrium. a) number of polymorphic loci used in analysis, b) mean number of alleles, c) from Merriam and Rentz 2010.

Site	Loci ^a	Alleles ^b	Mean H _e	Prob.	Est. size (ha) ^c
Goosenest	8	1.79	0.233	0.014	120
Flounce Rock	5	1.36	0.106	0.078	1.2
Miller Lake	8	1.79	0.224	0.014	20
Timbered Crater	7	1.86	0.206	0.055	2800
Wheeler Peak	7	1.71	0.148	0.234	30
Burney	11	2.14	0.261	0.16	600
Seiad	7	2	0.215	0.148	320
Hamburg	9	1.86	0.265	0.005	6.4

cone (serotinous) pines also with restricted distributions in California (mean 0.138–0.160, no SE given) (Millar et al. 1988). Compared to other long-lived woody species, H_e for Baker cypress was considerably higher than for 115 outcrossing gymnosperms (0.169), 241 outcrossing species (0.180), 50 species with gravity dispersed seed (0.178) and 57 outcrossing endemics (0.142) (Hamrick and Godt 1996). The relatively high level of genetic diversity is surprising as most of the known extant populations are limited in size, some considerably so (Table 1), and it is likely that the current distribution of the species is a remnant of a more widespread distribution. These results may indicate that it is only relatively recently that the distribution of Baker cypress has been reduced and populations have become isolated, and there has not been sufficient time for genetic diversity to be lost due to random genetic drift and/or inbreeding. Another possible explanation for the relatively high genetic diversity is that the serotinous cones are maintained on the tree with viable seed for many years before the heat from a fire causes them to open and release their seeds. As a result, each yearly cohort of cones represents an independent sample of the pollen cloud and therefore the general gene pool of the stand. Depending on phenological and climatic differences from year to year, the genetic composition of the half-sib seeds produced each year will vary. Therefore, when all of these seed are released after a fire, the resulting seed bank that will give rise to the next generation of trees is likely to have a broader genetic base than if it was only seed that were produced in a single year as it would be for most non-serotinous conifers. This pattern was observed in the canopy seed bank of two long-lived woody shrubs with serotinous cone-like structures in fire-prone areas of Australia (*Banksia spinulosa* Sm. and *B. hookeriana* Meisn.). In both of these plants, over several years genetic variation in the aerial seed bank quickly approached the maximal level detected in the parental population and contained 100% of the adult genetic diversity (Ayre et al. 2010, Barrett et al. 2005).

Despite the relatively high levels of genetic diversity within the Baker cypress populations, many of the populations had fixation indices greater than zero (Table 3) indicating a deficiency of heterozy-

gotes. A significant heterozygote deficiency was also reported in Tecate cypress by Truesdale and McCle-naghan (1998), which they attributed to high levels of non-random mating (inbreeding) and a likely Wahlund effect. The most common explanation for heterozygote deficiency here is as a result of inbreeding, which is likely for Baker cypress as its seeds are wingless and gravity dispersed, usually following fires which cause the opening of the serotinous cones. This is likely to result in regeneration of familial “islands” composed of nearly even-aged groups of individuals related as half-sibs. When these trees reach reproductive maturity this could result in matings among related individuals resulting in the observed heterozygote deficiency. Since Baker cypress generally only reproduces after fires, it is possible that a Wahlund effect is also contributing to the heterozygote deficiency if periodic fires that stimulate regeneration only impact limited parts of the population, resulting in uneven aged pockets where age classes are closely related to fire history (Merriam and Rentz, 2010). This may be especially true in the Miller Lake population as the density of Baker cypress in this stand is low and the individuals sampled were dispersed over a fairly large area.

Of the populations tested, the Flounce Rock population exhibited the lowest values for all measures of genetic diversity: fewer average alleles per locus, and lower percent polymorphic loci as well as lower observed and expected heterozygosity than other populations (Table 3). This population is the northernmost extent of the species range, so there is a possibility that being a peripheral population, it may have had lower genetic diversity to begin with. It has been suggested effective population size and gene flow should be highest at the core of a species range and lowest at the range margins (Eckert et al. 2008). Relationships between heterozygosity and latitude have been reported in many species of forest trees in western North America (Jaramillo-Correa et al. 2006 and references therein) and have been attributed to postglacial recolonization patterns. Although a negative relationship between heterozygosity and latitude was detected in Brewers spruce, Ledig et al. (2005) argued against the post-glacial recolonization theory for this species as the pattern they observed was irregular and suggested random genetic drift in

small populations rather than founder effects. They also argued that the postglacial recolonization theory is counter to the interpretation of Brewers spruce as a relic species that has persisted in the Klamath Region since the Miocene. The lack of any significant relationship between heterozygosity and latitude in Baker cypress similarly argues against the postglacial recolonization pattern and supports the idea that it also is a relictual species that was once more widespread.

Population Structure

Across all populations, Baker cypress showed moderate genetic structure. Genetic differentiation (F_{st}) (0.171) was very close to values reported for outcrossing species classified as having narrow ranges ($G_{st} = 0.169$) or endemic ($G_{st} = 0.179$), and for long-lived outcrossing species with gravity dispersed seed ($G_{st} = 0.177$), but higher than for outcrossing gymnosperms in general ($G_{st} = 0.073$) (Hamrick and Godt, 1992). However, due to the allele frequencies of the IDH locus, inclusion of the Flounce Rock population in the analysis had a strong influence on the overall level of population differentiation. With this population excluded, overall population differentiation was relatively low for such a highly fragmented species with geographically isolated populations (approximately 9% of the variation was among stands). This value was lower than both Monterey cypress (12% among stands) (Conkle 1987) and Tecate cypress (16% among stands) (Truesdale and McClenaghan, 1998), and also lower than the three California closed-cone pines (12, 22, and 13%) (Millar et al. 1988). The relatively low F_{st} (excluding Flounce Rock) for an endemic species with such a limited and disjunct distribution, and the lack of correlation between genetic and geographic distance suggests that the population structure in Baker cypress is due to random genetic drift in small, isolated populations. This may be further evidence that the extant populations of Baker cypress have only relatively recently contracted and become geographically isolated from each other. Raven and Axelrod (1978) propose that Tecate cypress was widespread during the Miocene and even into the Pliocene when summers were cooler and moister, colonizing serpentine areas as they became available. However, the widely distributed ecotypes disappeared as summer rains decreased during the early Pleistocene confining it to the serpentine areas as adaptation to the ultrabasic substrates removed them from competition from non-serpentine flora. In the more northern populations of its distribution, Baker cypress also occurs on serpentine soils, and in the southern populations it is often found on poorer volcanic soils. This supports the idea that the range contraction and population separation is of relatively recent origin. The current vegetation patterns likely developed only within the last 1500 to 3500 yr, and fossil evidence in general

indicates a recent contraction of all cypress species north of Mexico, which have highly fragmented distributions and generally occur on soils inhospitable to other species (Callahan 2013). Species with limited geographic ranges often occur in small isolated populations which are prone to genetic drift resulting in reduced genetic diversity within populations and as a result of limited gene flow, increased genetic differentiation among populations (Hamrick et al. 1992). If this is the case with Baker cypress, it does not appear that genetic drift has had sufficient time to diverge the populations more; however, the results from the BOTTLENECK analysis suggest that a reduction in effective population size during range contraction may have resulted in a loss of allele numbers and heterozygosity at polymorphic loci in some populations. This loss of allelic diversity resulted in an observed heterozygosity larger than the heterozygosity that would be expected from the observed allele number if the locus was at mutation-drift equilibrium (Piry et al. 1999).

The genetic divergence of the Flounce Rock population was evident from both the pairwise F_{st} analysis and the STRUCTURE analysis. However, the STRUCTURE analysis also revealed one genetic cluster for the Hamburg population and three genetic clusters among the other populations (Fig. 2). The Hamburg and Seiad populations are the closest geographically, yet the pairwise F_{st} value between these populations (0.091, Table 4) is the largest among all populations (excluding Flounce Rock). The Hamburg population harbored a private allele for the MDH1 locus, however, reanalysis with this locus excluded still yielded $K = 5$ in a STRUCTURE analysis, and the pairwise F_{st} with the Seiad population, while slightly lower (0.072) was still one of the highest (excluding Flounce Rock). While there appears to be some clustering of these populations based on allele frequencies, the pairwise F_{st} analysis shows that these populations are all more closely related to each other than any of them are to Flounce Rock, by an order of magnitude (Table 4). The clusters determined by STRUCTURE do not fit a geographic pattern and as a result it is likely that the allele frequency differences among these populations are due to drift. In these small populations with limited, if any gene flow among populations, drift could result in different alleles moving towards fixation for various loci among populations. A higher resolution marker with more loci may be able to resolve this, so while the F_{st} values do indicate a some genetic differentiation, the results of the STRUCTURE analysis should be viewed with caution in regards to grouping genetically similar populations.

Due to the limited size (both geographically and in the number of individuals) of most Baker cypress populations, even a relatively small fire could kill all of the adult trees in a given population. Like most serotinous conifers, Baker cypress regenerates prolifically after fire, so a single fire likely would not pose a

permanent threat to population persistence. However, if a subsequent fire kills all of the regeneration before it is able to reach reproductive age, this could lead to the extirpation of some of the smaller populations. These population genetic results show that there is genetic structure in the species most likely as a result of genetic drift. While some populations are genetically similar, there is no relationship between genetic and geographic distance, and therefore caution should be used if a decision is made to move seed among populations for purposes of reforestation.

CONCLUSIONS

Baker cypress is a rare, endemic tree species with small, highly isolated populations. Despite the high degree of geographic isolation among populations, levels of genetic diversity are higher than similar species made up of isolated populations. It has a moderate degree of genetic structure among populations, and most populations show a slight heterozygote deficiency, but our results indicate that the current population structure of the species is likely a fairly recent reduction from a formerly widespread distribution with drift leading to genetic differentiation. Baker cypress is a fire adapted species and it requires fire for regeneration. Recent fires in some stands have resulted in high levels of regeneration, however some of the smallest populations show significant impacts from fire exclusion, including high stand density, slow growth, and little or no regeneration. Introduction of fire to these stands is likely to be beneficial to these stands in the long run, but if regeneration fails for some reason, it could mean the catastrophic loss of an entire population. Seed collection and storage would be one form of insurance to prevent the loss of the genetic base of any population. The Moonlight Fire burned with a range of intensities through the Baker cypress population at Mud Lake, causing high mortality rates in some areas, but leaving other portions of the population (Merriam and Rentz 2010). While this resulted in substantial cypress regeneration after the fire (Merriam and Rentz 2010), a stand-replacing fire is not likely to be a suggested management recommendation for other small populations of Baker cypress that are currently in poor condition, such as the population at Flounce Rock. Actions to restore the condition of this, and other populations are likely to include treatments such as thinning, pile burning, and/or low intensity underburns, along with cone collections and planting seedlings (M. Wineteer, personal communication). Our results indicate that populations do differ genetically to some degree and these differences are not related to geography, so it is not advised to move seed among populations, even among ones that are geographically close. In addition, our results do not address potential differences in local adaptation, which could be assessed through a common garden test. It is

recommended that seed be collected and used within the stands from which it originated and in populations most in need of restoration, activities be carefully planned to simulate natural burning to encourage natural regeneration, with the option to plant seedlings raised from the collected seed should natural regeneration fail. Thinning some of the denser areas of these stands, with some burning of both slash and duff may simulate a low intensity fire, providing the heat needed to open cones on the trees and release seeds and the bare mineral soil needed for these seeds to germinate.

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NOT ALL PLANT TAXA DISPLAY TYPICAL LATITUDE-PROPAGULE SIZE GRADIENTS: A CASE STUDY IN *ARCTOSTAPHYLOS* L. (ERICACEAE)

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ABSTRACT

Proposed causes of the latitudinal-propagule size gradient invoke differences among biome structures or seed dispersal syndromes. Latitudinal seed-size gradients so far have been predominantly investigated using entire floras, prompting the question of whether such trends exist at smaller scales. Here we consider effects of latitude and elevation on fruit size between and within species in *Arctostaphylos* L. (Ericaceae), a zoochorous, primarily Californian chaparral shrub genus. We measured fruit size, strongly correlated with seed size in *Arctostaphylos*, in three species of this genus from multiple localities in the Sierra Nevada foothills, and conducted a character analysis of fruit size across the genus using the standard California flora plant manual (Baldwin et al. 2012). Across the genus we found a weak negative correlation between fruit size and latitude (0.0026 mm diameter/km) and a weak positive correlation between fruit size and elevation (1.3 mm diameter/km). AIC indicates that these trends are not explained by autocorrelation between fruit size and other variables such as maximum plant size. By contrast, intraspecific field data revealed a positive relationship between fruit size and both elevation and latitude. Propagule size gradients within *Arctostaphylos* oppose those reported for angiosperms globally. This contrast may result from uniformity of fruit structure and animal dispersal, disturbance ecology of chaparral, or local precipitation gradients characteristic of Mediterranean-type climates. Studies of propagule size gradients within taxa can uncover ecological mechanisms behind this trend that remain obscure at global scales.

Key Words: *Arctostaphylos*, California, elevation, fruit size, latitude, propagule size, Sierra Nevada foothills.

Variation in seed size influences a large number of ecological and physiological processes in plants (Kitajima and Myers 2008, Moles and Leishman 2008), such as trade-offs between seed mass and seed number or seedling survival. Seed size simultaneously may reflect multiple selective processes acting on the life history of plants. Here we examine patterns found within *Arctostaphylos* L. (Ericaceae; manzanita), which has a large number of species that vary greatly in fruit size. Fruits in this genus can contain 2–10 seeds that are variously and sometimes strongly fused into a group, which at maturity are surrounded by a mealy mesocarp and a thin exocarp. Generally, *Arctostaphylos* fruit are the dispersal propagule, dispersing seeds within as a single unit. Because seed and fruit mass are strongly correlated ($P = 0.01$, $\rho = 0.70$; Moore 2014, Parker 2015), here we use fruit size as a surrogate for seed size.

Global geographic trends in the distribution of ecological traits, such as the latitudinal seed size gradient in plants (e.g., Moles et al. 2007), compile together multiple evolutionary and ecological rela-

tionships within individual taxa that occur over smaller spatial and ecological scales. By restricting our study to the genus *Arctostaphylos*, we consequently hold a number of aspects constant that have been used to explain global patterns, such as variation in light environments, dispersal vectors, growth form, and vegetation type. We hope that these local patterns provide insight into significant trait variation seen within genera that have radiated into a variety of soil and climate conditions. By examining latitudinal and related gradients in fruit size in *Arctostaphylos*, we also can address questions of whether and how the global gradient represents a model for genera restricted to regions along that gradient or whether the global trends are scale-dependent. Does fruit size in all genera respond equally to the pressures of latitude and elevation?

Several hypotheses propose to explain latitudinal gradients in seed size (Moles et al. 2007). In our study, we test the following two: (1) Growth form correlates with seed size with woody plants having larger seeds than herbs (Lord et al. 1997, Wright et

al. 2007); tropical ecosystems contain a higher percentage of trees that typically have large seeds. (2) Vertebrate dispersal of fruits is considered to be more available in the tropics than the temperate zone, thereby freeing propagules from the size constraints necessary for effective wind dispersal (Wilson et al. 1989, Lord et al. 1997). In the context of *Arctostaphylos*, the genus varies considerably in overall plant size, which enables the hypothesis that larger plants have larger propagules (#1 above) to be tested directly. Furthermore, all species of *Arctostaphylos* are woody and benefit from animal-mediated dispersal. Therefore, if latitudinal propagule-size gradients can be attributed to differential frequency of dispersal mechanism (#2 above), then there should be no latitudinal gradient in *Arctostaphylos*.

In addition to the above two hypotheses, two more have been proposed that we do not here examine (Moles et al. 2007): (3) Seedlings in closed-canopy tropical forests require extra leaf surface to collect sufficient light in a shaded understory (Salisbury 1942). Finally, (4) lower latitude climates are more favorable to growth, including longer growing seasons and higher net primary productivity, which may also enable the growth of larger seeds. Climate and vegetational profiles of the California Floristic Province do not follow the global latitudinal gradients in either canopy closure or net primary productivity, due to the fact that the southern parts of California are drier, if warmer, than the northern parts, and the moisture profile also is tightly associated with elevation. *Arctostaphylos* is often associated with trees in habitats where it is found, but in most cases these trees do not form a closed canopy. As a consequence, testing either of the hypotheses that canopy closure or net primary productivity is outside of the scope of what can be confidently done using this genus.

Our working hypothesis is that fruit size variation in *Arctostaphylos* should match global patterns. Holding dispersal agents constant tests whether environmental gradients found within the California Floristic Province have an impact different from dispersal processes. Studies of seed size as a function of latitude over small spatial scales have supported global patterns, using as a model both the regional California flora (Baker 1972) or elsewhere (Mazer 1989). However, because the moisture gradient in California runs from south (arid) to north (mesic), contrary to global trends, we might as an alternative hypothesis anticipate a canopy-closure or drought-induced primary productivity gradient running from south to north as well. A positive elevational gradient in rainfall also exists in California, but Baker found that seed size at the floristic scale decreases with increasing elevation. If fruit size in *Arctostaphylos* runs contrary to the trends found by Baker for the California flora as a whole, then some aspect of the ecology of the genus is different from the processes responsible for the global propagule-size gradient.

We make similar predictions about the distance of a species from the coast, as more inland environments in California are drier and have more extreme temperatures. Because the coastline in California is largely north-south, longitude provides a rough estimate of coastal difference and continental climate for individual field samples.

Patterns in fruit size variation within *Arctostaphylos* should help evaluate alternative hypotheses concerning underlying influences in fruit-size geographic gradients. Disagreement in patterns between *Arctostaphylos* and in the global flora would imply that global-scale patterns are driven by characters that are invariant within *Arctostaphylos*, such as animal-dispersal or fire-stimulated persistent seed. Alternatively, a pattern consistent with the global pattern would suggest that ecological or evolutionary characteristics that vary among species drive the gradient. A lack of statistical pattern might suggest fruit variation within *Arctostaphylos* would be of stochastic or phylogenetic origin. *Arctostaphylos* species are thought to be chiefly dispersed by scatter-hoarding rodents, although other animals, including bears are known to disperse *Arctostaphylos* seeds (Parker 2015, Moore and Vander Wall 2015).

METHODS

Literature Floral Survey and Meta-Analysis of *Arctostaphylos*

There were two phases of data collection and analysis in this study. Because all species of *Arctostaphylos* are found naturally in California, we first used Baldwin et al. (2012) to compile a database of fruit size, elevation range, and regeneration strategy (whether species are able to resprout from burls) for all taxa ($n = 95$) in the genus. Baldwin et al. do not give latitudinal limits for any species, but geographic distributions are described in narrative form. Latitudes were inferred by matching the narrative descriptions in Baldwin et al. to geographic maps and software, primarily Google Earth (earth.google.com, accessed on 1 November 2008).

For each species, thirteen continuous variables were collected: minimum, median, and maximum values for fruit size, latitudinal range, altitudinal range, and distance from coast, plus maximum height at maturity. We chose to include both minimum and maximum values for the aforementioned variables because each may represent different endpoints in the factors influencing propagule size. For example, a species with a large latitudinal range might have large fruits at the southern limit of its range, but small fruits at the northern edge, which would not be recovered using a single latitude or fruit size variable. Baldwin et al. (2012) provides only qualitative information on the sizes of burls, so taxa were scored for presence (1) or absence (0) of burls. Taxa that sometimes have burls were scored as having burls for the purpose of this study. Taxa within

Arctostaphylos were resolved to the finest scale resolution possible; if a species was divided into subspecies as per Baldwin et al. (2012), then subspecies were used as data points. While this may introduce bias of phylogenetic non-independence of data, the amorphous distinction between biological species for much of the genus (Boykin et al. 2005) precludes useful phylogenetic controls for this analysis. Fruit volume was calculated from diameter under the assumption of a spherical fruit, which is generally a good assumption for *Arctostaphylos*. For those species with ellipsoid fruit, volume was calculated based on fruit dimensions assuming an oblate spheroid geometry.

Intraspecific Field Sampling

Fruits from three species of *Arctostaphylos*, *A. manzanita* Parry (common manzanita), *A. patula* Greene (greenleaf manzanita), and *A. viscida* Greene (whiteleaf manzanita) were collected in the western Sierra Nevada during the summer and early fall of 2008. Taxa were chosen for their wide geographic and elevational ranges, allowing for a meaningful comparison of fruit size and environmental variables, and for the accessibility of obtaining fruits. Fruit physical dimensions were measured and collection location recorded using GPS (latitude, longitude, elevation). Five fruits were collected and measured from each of the 330 plants sampled. Fruits collected from each plant were those that appeared healthy and well-developed, comparable in size to dropped fruit from previous seasons and not underdeveloped relative to other fruits on the plant. This choice might result in a bias toward larger fruit sizes; however, this should not affect trends within species because such biases would be systematic across study sites. The study area included accessible portions of chaparral communities, from the southern range of the study area northward, along California highways from 37°48' to 40°30' latitude and from 170 to 2500 m elevation.

Data Analysis

For each fruit size variable (minimum, maximum), we constructed generalized linear models using the generalized linear model (glm) function in R (R Core Development Team 2016) for each of the eight explanatory variables (height, burl presence/absence, max., min. and range of elevation, and max., min., and range of latitude). In addition to analyzing each explanatory variable individually against fruit size, we also analyzed each pair of explanatory variables against fruit size, as well the following blocks of variables: plant height with all elevation variables, plant height with all latitude variables, all elevation variables with all latitude variables, burl presence/absence with all elevation variables, and burl presence/absence with all latitude variables, and finally all eight variables together in one model. In

all models examining more than one explanatory variable, we also examined all relevant two-way interactions, but did not examine interactions more complex than two-way. We then used Akaike's Information Criterion (AIC) within R to select the best among the models that we tested.

In the intraspecific study, mean geometric fruit volume at maximum size of development was calculated for each plant sampled for the three (*A. manzanita*, *A. patula*, and *A. viscida*). For these species, we ran all combinations of linear models of fruit size as a function of latitude, longitude, and elevation, but without interaction terms between explanatory variables. As with the intraspecific data, we then used AIC to select the best model.

RESULTS

Interspecific Character Analysis

AIC analyses indicate that the best model explaining minimum fruit size includes both the height of the plant at maturity and also the distance that the species lives from the coast (Table 1, Fig. 1). Plant height is positively correlated with minimum fruit size. Relationships between minimum fruit size and coastal distance are remarkably flat, but are slightly positive for minimum distance from coast and slightly negative for maximum distance from coast. The null model in which minimum fruit size is unaffected by any of the variables under scrutiny is roughly a factor of 10⁶ worse than the best model using plant height and distance from coast.

The best model explaining median fruit size includes only median latitude, which correlates negatively with fruit size (Table 1, Fig. 2). However, this best model is not more than twenty times as likely as eight other models, including the null model. Most, but not all, of these other models do include latitude as a covariate, however.

The best model explaining maximum fruit size includes all of the explanatory variables that we measured (Table 1, Fig. 3). In this model, fruit size decreases with increasing latitude, and increases slightly with increasing elevation and with fruit size. Distance from the coast seems to correlate positively with maximum fruit size, but statistically this effect may be dominated by a few very wide-ranging species. The null model in which maximum fruit size is independent of both plant height and minimum latitude is roughly 10⁷ times worse.

Intraspecific Field Sampling

Summary statistics for our field samples are presented in Table 2. The best model explaining fruit size in *A. manzanita* includes elevation, latitude, and longitude (Table 3), which is more than 10⁴ times more likely than a null model. Regression on these variables reveals that fruit size increases with both

TABLE 1. AIC-based model selection results explaining fruit size in *Arctostaphylos* based on Baldwin et al. (2012). The best model (lowest AIC) score is shown for minimum, median, and maximum fruit size, as well as selected other models including the null model and all models at least 5% as likely as the best model, and the best runner-up model less than 5% as likely as the best model. “Inland” in models refers to the distance from each *Arctostaphylos* taxon to the nearest coast.

Dependent variable	Model	AIC score	Akaike weight (relative likelihood)
Min. fruit size	Plant height + min. inland + max. inland	986.84	1
	All parameters	994.15	0.026
	Null	1015.86	5×10^{-7}
Med. fruit size	Med. latitude	−256.23	1
	Med. latitude + med. inland	−253.68	0.28
	Med. latitude + burl	−253.64	0.27
	Burl	−253.39	0.24
	Plant height + med. latitude	−253.33	0.24
	Med. elevation + med. latitude	−252.44	0.15
	Null	−252.24	0.14
	Med. inland	−250.51	0.06
	Plant height	−250.44	0.06
	Med. elevation	−250.36	0.05
	Plant height + burl	−249.86	0.04
Max. Fruit size	All parameters	955.23	1
	Plant height + min. inland + max. inland	959.47	0.12
	Plant height + maximum latitude	965.75	0.005
	Null	988.09	7.3×10^{-8}

elevation (Fig. 4; Table 4) and latitude (Fig. 5; Table 4), and decreases moving eastward (Fig. 6; Table 4). The best model explaining fruit size in *A. viscida* includes both latitude and elevation; however, several other models have likelihoods at least 5% that of the best model, some involving longitude (Table 3). This model is over 100 times more likely than the null model. Using regression, fruit size in *A. viscida* increases with elevation (Fig. 4; Table 4) and with latitude (Fig. 5; Table 4). The regression line of best fit suggests that fruit size in *A. viscida* appears to decrease moving eastward (Fig. 6; Table 4) as in *A. manzanita*, but the effect is very small if it is real. The best model explaining fruit size in *A. patula* includes longitude and no other variables. However, all seven other models are at least 5% as likely as the best model, including the null model (Table 3). Regression lines of best fit suggest that fruit size in *A. patula* decreases with elevation (Fig. 4; Table 4), increases with latitude (Fig. 5; Table 4), and decreases moving eastward (Fig. 6; Table 4), if these relationships are real.

DISCUSSION

Initially, we set out to test two hypotheses regarding the latitudinal (and Baker’s elevational) propagule-size gradients. The hypothesis that larger plants tend to have larger propagules than smaller plants is largely borne out: plant height is correlated with both minimum and maximum fruit size in the best model, and the correlation is strongly positive. The hypothesis that a latitudinal gradient in dispersal mode is responsible for the latitudinal gradient in

propagule size does not agree with our data, because this hypothesis predicts that there should be no latitudinal gradient in the universally animal-dispersed *Arctostaphylos*. Despite this, median fruit size correlates negatively with latitude at an interspecific level in the genus, while at least *A. manzanita* and possibly *A. viscida* and *A. patula* as well see fruit size correlating positively with latitude. In all of these trends that we observed, the effect sizes are not large; most of the variation in fruit size is not determined by elevation, latitude, or any other variable that we investigated. However, because we are investigating a geographically small area relative to the global scale of the latitudinal seed size gradient, we should not expect the effect sizes to be large over this small scale. While *Arctostaphylos* displays the same fruit size trends with latitude at both the global (e.g., Moles et al. 2007) and floristic (Baker 1972) scale, these trends are not replicated on the intraspecific level in any of the three species tested, and *A. manzanita* and possibly *A. viscida* and *A. patula* as well exhibit the opposite trend. Furthermore, all measures of *Arctostaphylos* that show a statistically significant trend show a positive correlation between fruit size and elevation, in contrast to Baker’s (1972) findings for the California flora as a whole. We find the variation in the fruit size/latitude relationship between different taxonomic levels and between individual species somewhat surprising. We cannot tell from these data whether the differences in intraspecific trends result from these three species being anomalous, or if fruit size gradients typically change between species and as a function of taxonomic scale. *A. manzanita*, *A. viscida*, and *A. patula* are all widespread in Califor-

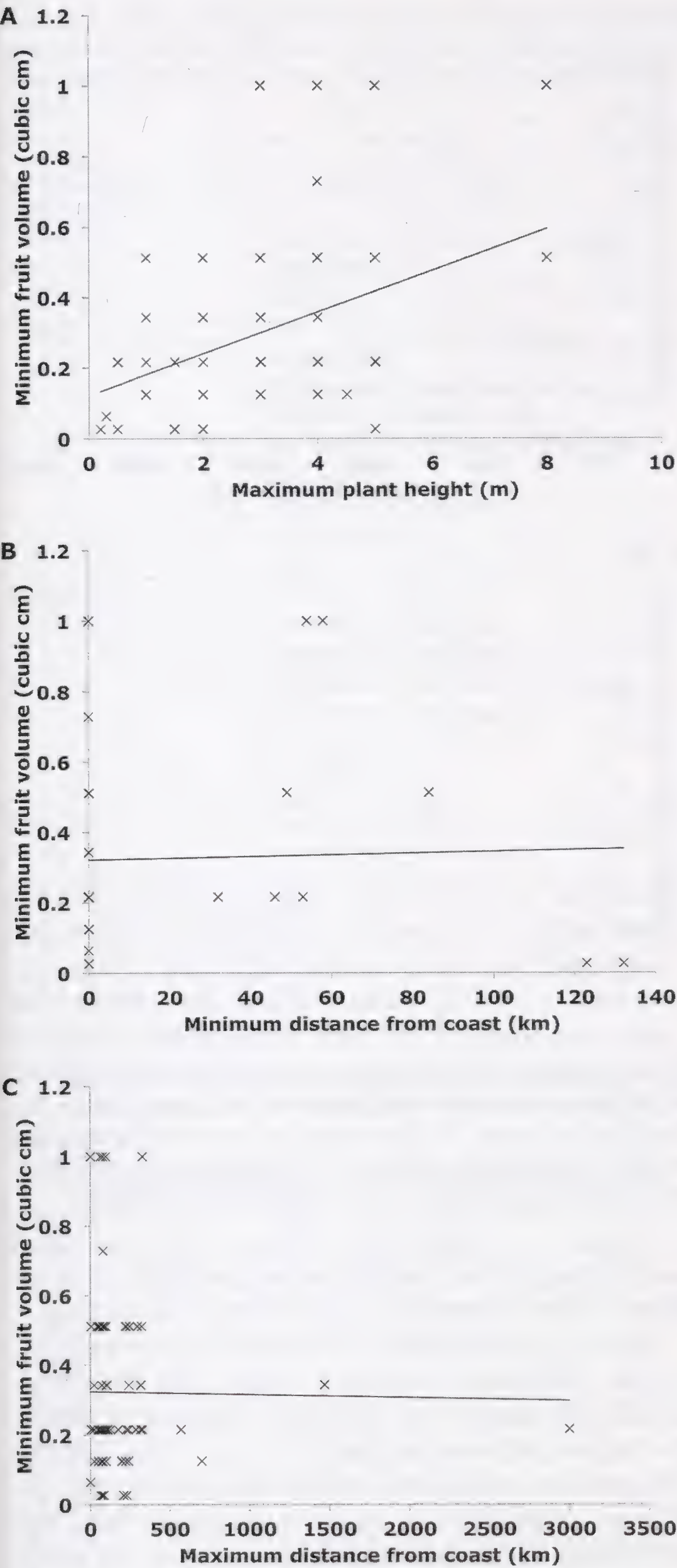


FIG. 1. Graphs of minimum fruit size (in cubic centimeters) in *Arctostaphylos*, based on data from Baldwin et al. (2012). Each data point represents a species. A. Minimum fruit size as a function of maximum plant height. B. Minimum fruit size as a function of the closet proximity of a species' range to the ocean. C. Minimum fruit size as a function of the greatest distance a species occurs from an ocean. AIC analysis (Table 1) indicates that all three of these explanatory variables together make for the best prediction of minimum fruit size.

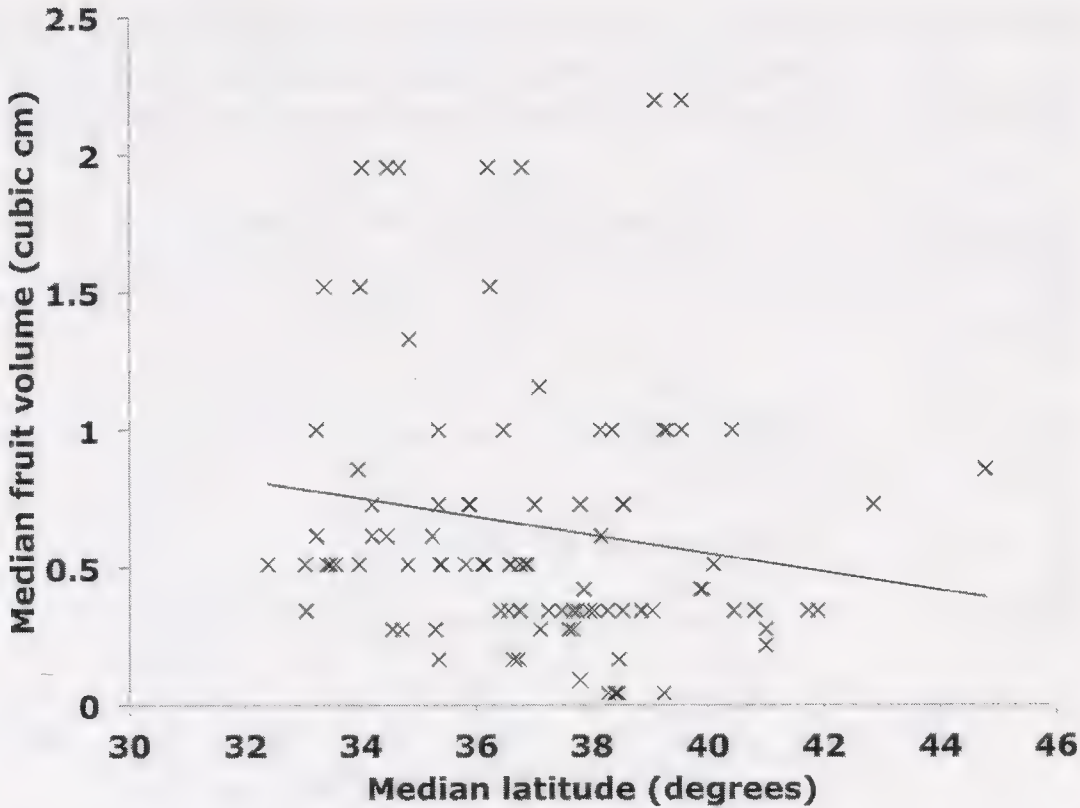


FIG. 2. Graph of median fruit size (in cubic centimeters) in *Arctostaphylos* as a function of median latitude at which a species occurs. Each data point represents a species. While AIC analysis (Table 1) suggests that the best model explaining median fruit size includes only latitude, several other models using other variables (including a null model) are not statistically worse by a 5% likelihood cutoff.

nia, in contrast to the local endemics ($n = 65/95$ from our character analysis) with restricted ranges composing the majority of species in the genus. Because most of the larger-seeded species with broad geographic ranges are found in southern California, examining intraspecific patterns in these species would test the consistency of the reversal of the correlation at the species scale.

Because the Mediterranean-climate of California becomes moister at higher latitude, the moisture pattern represents a localized reversal of the global aridity gradient from equator to pole. Further, a large number of species in *Arctostaphylos* are also local endemics that occur near the central and northern Pacific coast of California in moderate maritime climates. Thus, if fruit size in *Arctostaphylos* increases with moisture and not temperature, we would predict a positive relationship between fruit size and both latitude and elevation. At the interspecific level, maximum fruit size increases with elevation, which matches a moisture gradient. We recovered the same pattern in *A. manzanita* and possibly the other two species as well. In contrast to a global moisture correlation, Baker (1972), who examined seed mass (not fruit volume), found significant differences between seed mass in moist versus arid communities in the California flora. In his studies, seed mass increases in more arid communities. In all gradients that we examined, fruit size in *Arctostaphylos* either runs counter to the trends that Baker found or exhibits no significant trend, implying that the processes responsible for seed mass gradients in California as a whole do not determine what happens in *Arctostaphylos*.

Phylogeny is known to be relevant to propagule size but we did not control for it (Moles et al. 2005). The phylogeny of *Arctostaphylos* is currently unre-

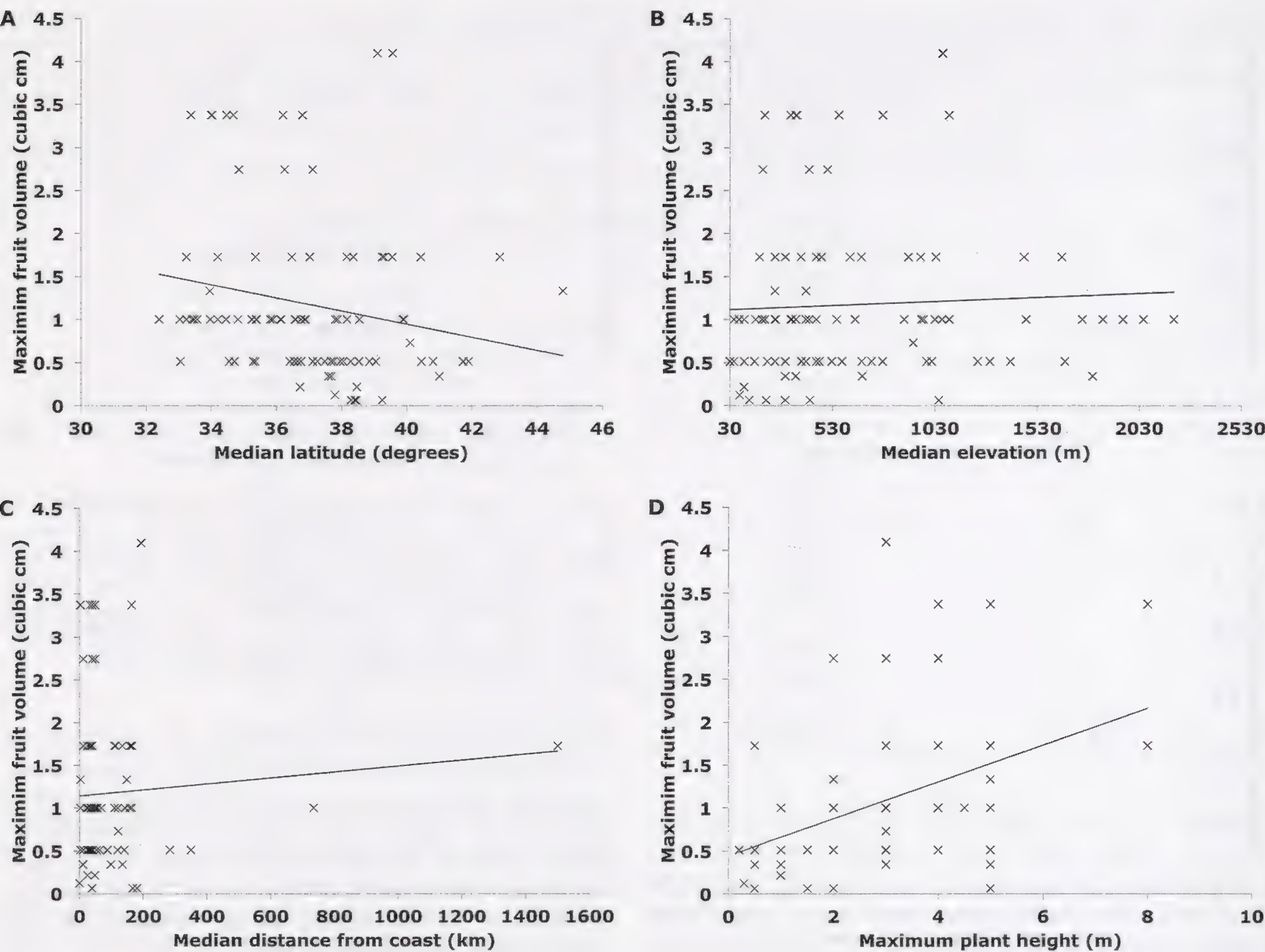


FIG. 3. Graphs of maximum fruit size (in cubic centimeters) in *Arctostaphylos*. Each data point represents a species. A. Maximum fruit size as a function of median range of latitude. B. Maximum fruit size as a function of median elevation. C. Maximum fruit size as a difference of median distance of a species from an ocean. D. Maximum fruit size as a function of plant height. AIC analysis (Table 1) suggests that while the best model explaining maximum fruit size includes all of these variables, a model including only plant height and distance from an ocean is not worse with a 5% likelihood cutoff.

solved, but appears to be divided into two large clades (Boykin et al. 2005, Wahlert et al. 2009); when the phylogeny is better resolved, controlling for it may account for some currently unexplained variation. Next, while individual species of *Arctostaphylos* possess characteristic sizes, almost all are shrubs ranging from 0.5 m to about 3.0 m in height. *Arctostaphylos uva-ursi* (L.) Spreng., for example, as an alpine or coastal species, is smaller, but its removal as an outlier does not affect the strength of correlations. As a consequence, the variation in height within a flora containing both trees and herbs will be greater than that contained solely within *Arctostaphylos*; thus, relationships between height

and propagule size would not be predicted to be as large within the genus as in the flora as a whole.

Most of the diversity of *Arctostaphylos* is endemic to the California Floristic Province, but biomes in California range from subtropical deserts to Mediterranean chaparral to alpine tundra. If the latitudinal gradient were weak merely because we did not sample enough of a latitudinal range, then the altitudinal gradient would be expected to be much stronger, which it is not. Selection for traits involved in fruit size gradients may therefore be weak in *Arctostaphylos*. One potential explanation for weak, nonexistent, or even reversed gradients is that the primary driver of the latitudinal gradient, namely

TABLE 2. Summary statistics of our intraspecific field study of *Arctostaphylos* fruit size as a function of geographic variables. Fruit size is in units of cubic centimeters. The abbreviation “SD” stands for standard deviation.

Taxon	Fruit size mean	Fruit size SD	Elevation mean	Elevation SD	Latitude mean	Latitude SD
<i>Arctostaphylos manzanita</i>	0.72	0.27	737	334	39.9	0.7
<i>Arctostaphylos patula</i>	0.55	0.13	1777	244	39	0.8
<i>Arctostaphylos viscida</i>	0.21	0.05	875	267	39	1

TABLE 3. AIC-based model selection results explaining fruit size in three species of *Arctostaphylos* from field samples. The best model (lowest AIC) score is shown for each of the three species, as well as selected other models including the null model and all models at least 5% as likely as the best model, and the best runner-up model less than 5% as likely as the best model.

Taxon	Model	AIC score	Akaike weight (relative likelihood)
<i>A. manzanita</i>	Elevation + latitude + longitude	−0.68	1
	Elevation	7.76	0.015
	Null	19.6	3.9×10^{-5}
<i>A. patula</i>	Longitude	−125.79	1
	Latitude + Longitude	−124.76	0.6
	Elevation + Longitude	−123.8	0.37
	Elevation + latitude + longitude	−123.15	0.27
	Latitude	−122.94	0.24
	Elevation + latitude	−121.99	0.15
	Elevation	−121.4	0.11
	Null	−120.8	0.083
<i>A. viscida</i>	Elevation + latitude	−345.84	1
	Latitude + longitude	−345.7	0.94
	Elevation + latitude + longitude	−345.42	0.81
	Elevation + longitude	−344.8	0.59
	Elevation	−341.73	0.13
	Latitude	−337.52	0.016
	Null	−336.06	0.0075

dispersal mode, has been controlled by the study system. Nonetheless, fruit volume in *Arctostaphylos* as a genus varies over three orders of magnitude (calculated from Baldwin et al. 2012). Local environmental gradients in addition to regional climate patterns, such as those caused by slope, aspect, or soil type, may be responsible for most of this variation.

We suggest that ecological aspects of *Arctostaphylos* (wildfire, drought, persistent seed banks, numerous species endemic to edaphic environments)

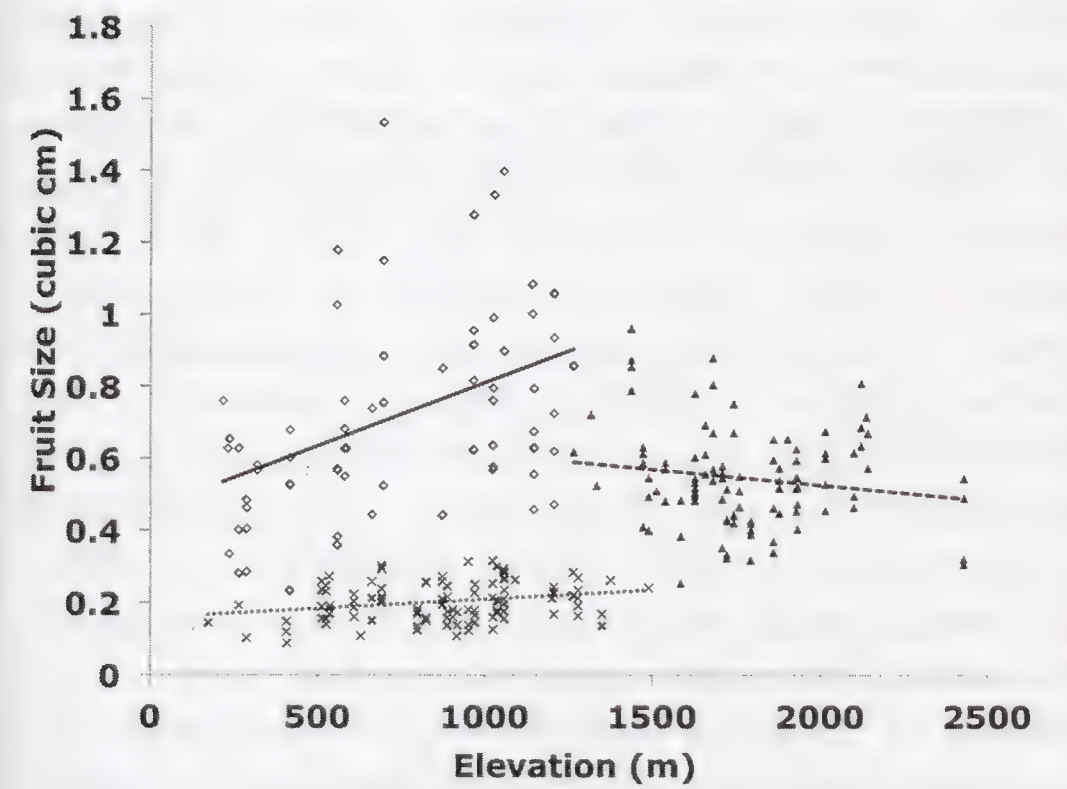


FIG. 4. Regressions of fruit size as a function of elevation in field samples of three species of *Arctostaphylos*. Open diamonds and solid lines represent *A. manzanita*, filled triangles and dashed lines *A. patula*, x symbols and dotted lines *A. viscida*. Summary statistics for these distributions are available in Table 2, and AIC analysis of statistical significance in Table 3.

may be driving the observed patterns. Species living in edaphic environments could be predicted to have less resources with which to make fruits, and so their fruits would be smaller. High-elevation environments in California are generally dominated by granitic soils, with the result that edaphic species at low elevation may account for the interspecific fruit size gradient in the genus. However, *A. manzanita* does not grow in edaphic environments, yet still increases fruit size with elevation, so edaphic environments alone cannot explain the elevational fruit size gradients in *Arctostaphylos* at all levels. *Arctostaphylos* differs from most other plant genera in its response to wildfire. In the Sierra Nevada, *Arctostaphylos patula* resprouts following fire from burls, as do some *A. manzanita* (only subsp. *roofii* [Gankin] P. V. Wells), but not *A. viscida* (Baldwin et al. 2012). Yet, all species of *Arctostaphylos* live in fire-prone areas, and all establish new individuals from persistent seed banks in a high-light, droughty, post-fire environment. Variation in fruit size (and seed size) may reflect the geographic climate variation as it influences the difficulty in establishing seedlings in the post-fire environment. Drought should select for larger fruit (seeds) so that seedlings have a better opportunity to establish root systems deep enough to survive a more severe summer drought. While elevation relaxes the moisture deficit, growing season length is reduced and large fruit size may be favored by selection to provide seedlings more of chance to establish prior to winter storms and burial in snow. Because rodents are found throughout the elevational gradient of *Arctostaphylos* and because they are the chief

TABLE 4. Linear regression coefficients for relationships between fruit size and latitude, elevation, and longitude for *Arctostaphylos manzanita*, *A. patula*, and *A. viscida*. Statistical significance of these relationships addressed via AIC-based model selection, the results of which are presented in Table 3.

Taxon	Explanatory variable	Slope	R ²
<i>A. manzanita</i>	Elevation	0.4 cm ³ /km	0.18
	Latitude	0.84 cm ³ /degree	0.045
	Longitude	0.13 cm ³ /degree	0.13
<i>A. patula</i>	Elevation	−0.09 cm ³ /km	0.025
	Latitude	0.036 cm ³ /degree	0.041
	Longitude	0.055 cm ³ /degree	0.055
<i>A. viscida</i>	Elevation	0.05 cm ³ /km	0.068
	Latitude	0.0097 cm ³ /degree	0.034
	Longitude	0.0089 cm ³ /degree	0.0089

dispersal agents, differences in dispersal ecology are unlikely to be responsible for the gradients in fruit size that we observed. As such, one might predict that there would be an elevation at which a maximum fruit size is attained, as supported by *A. manzanita* being below this maximum, but *A. viscida* and *A. patula* straddling or above it. If so, then similar patterns with elevation could be obtained from southern California inland manzanitas. Moore (2014) addressed in similar questions in other species of *Arctostaphylos* and *Ceanothus* L. and found that *Arctostaphylos* does not conform to global patterns of fruit and seed size, with which our results agree.

We are aware of few studies that have examined propagules at a scale smaller than the flora most

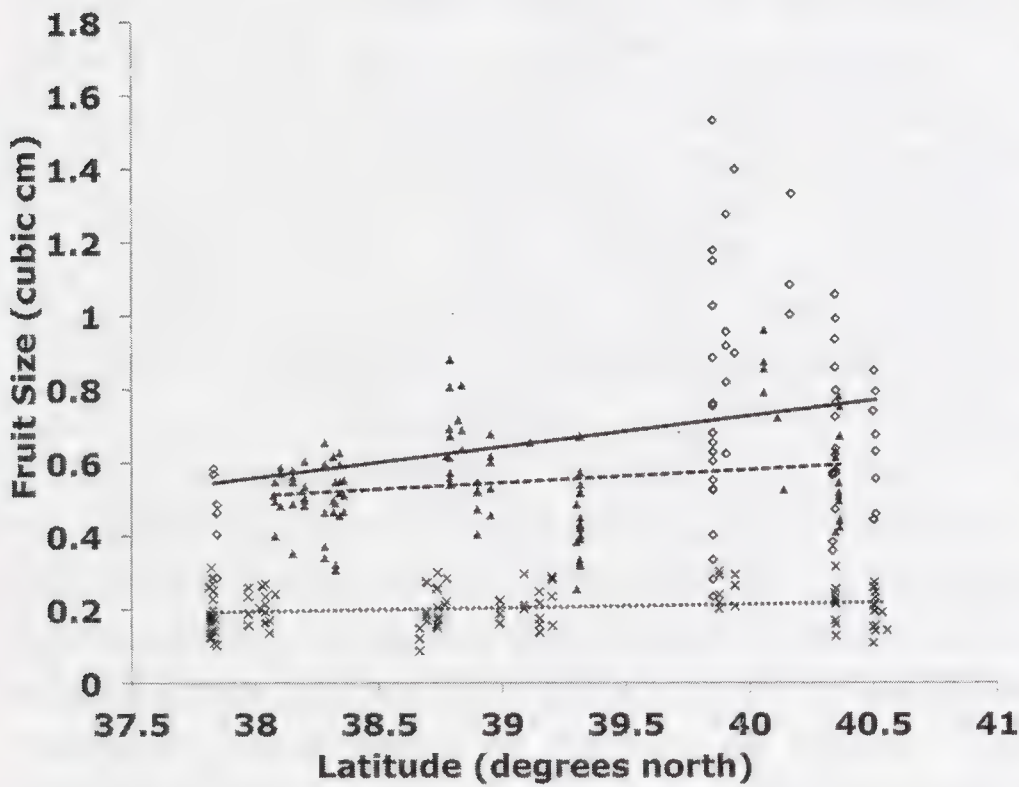


FIG. 5. Regressions of fruit size as a function of latitude in field samples of three species of *Arctostaphylos*. Open diamonds and solid lines represent *A. manzanita*, filled triangles and dashed lines *A. patula*, x symbols and dotted lines *A. viscida*. Summary statistics for these distributions are available in Table 2, and AIC analysis of statistical significance in Table 3.

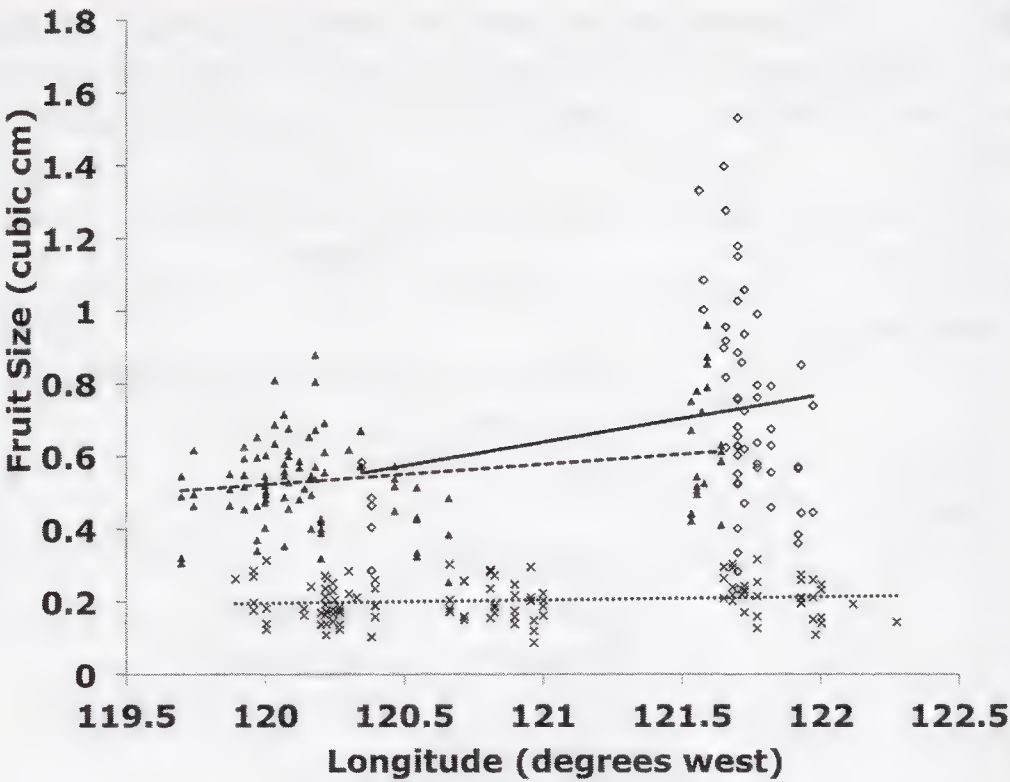


FIG. 6. Regressions of fruit size as a function of longitude in field samples of three species of *Arctostaphylos*. Open diamonds and solid lines represent *A. manzanita*, filled triangles and dashed lines *A. patula*, x symbols and dotted lines *A. viscida*. Summary statistics for these distributions are available in Table 2, and AIC analysis of statistical significance in Table 3.

have been meta-analyses using published range sizes. Investigating fruit size patterns at taxonomic scales lower than global or regional floras indicates that while patterns may match or contrast with global patterns, underlying mechanisms are likely to be driven by ecological issues specific to the taxon. In this study we tried to hold constant a number of ecological or evolutionary processes thought to drive the global pattern. Our results indicate that *Arctostaphylos* does not consistently abide by the same patterns as the global flora. Murray et al. (2003, 2004) investigated seed size in Australian *Glycine* L. (Fabaceae), and found similar results to ours in *Arctostaphylos*: seed mass increases with decreasing latitude and with increasing aridity, which they interpret as being related to greater availability of photosynthate from higher light intensity. However, they also found that seed mass decreases with latitude within species as well as among species, in contrast with what we found. Moore (2014) studied *Ceanothus* in California, a genus with similar ecological characteristics to *Arctostaphylos*. Additionally, in Australia and South Africa, a number of additional genera (especially in the Proteaceae) offer additional opportunities to test global patterns at a lower taxonomic scale in fire-prone Mediterranean-type climates. Ultimately, many taxa will need to be studied in order to determine how propagule size variation is affected by latitude, elevation, and other macroecological variables.

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VARIATIONS IN SAGUARO CACTUS (*CARNEGIEA GIGANTEA*) SPINE LENGTH IN WET AND DRY PORTIONS OF THEIR RANGE

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ABSTRACT

I sampled spine length in the keystone *Carnegiea gigantea* (Engelm.) Britton & Rose in two northern Sonoran Desert populations where this species is ultimately limited by cold temperatures. I compared spine length (1) between two sites, (2) on north versus south facing sides of plants, (3) by level of shading, (4) by proximity to other potential conspecific competitors, and (5) by presence near sources of surface runoff from which all their water is obtained. Spine length increases, providing thermal protection and reducing photosynthesis, where temperature is more extreme (winter and summer) and where conditions are more xeric. The difference in spine length by direction on plant was much smaller at the more extreme site. Clumped plants that necessarily had increased competition for water but also greater shading were split; at the more extreme site, greater spine shading occurred where water competition was fiercest, while at the less extreme site, greater shading occurred in association with sunlight rather than water.

Key Words: Cactus, *Carnegiea gigantea*, plant-climate relationships, Sonoran Desert.

The benefits of spines are extensive and cacti invest substantial resources to produce them (e.g., Nobel 1988). Our understanding of the function of spines, particularly with regard to climate, is still limited (Steenbergh and Lowe 1983). A variety of explanations have been offered for the purpose of cactus spines. Benefits include protecting water stores from animals (e.g., Stelfox and Vriend 1977, Steenbergh and Lowe 1983), elevating loose cladodes above the hot ground surface (Nobel et al. 1986), water absorption (see Nobel 1983), and protecting the apical meristem from winter freezes by elevating minimum temperatures (Lewis and Nobel 1977, Nobel 1980a, Nobel and Bobich 2002). Spine length and density are related to climate conditions, with increases in length and density observed in a species in more extreme climates and microsites (Yeaton et al. 1980, Nobel 1980b). Cactus surface temperatures are more moderate on spine-covered ridges than on the unspined furrows (Drezner 2011). Spines reflect solar radiation and provide stem shading, reducing maximum surface temperatures in summer (Lewis and Nobel 1977, Nobel 2002). However, spines reduce photosynthesis by reducing CO₂ uptake (Nobel 1977, Lewis and Nobel 1977), which decreases productivity and may be disadvantageous (Nobel 1983), including slowing growth (Nobel 1988).

The keystone cactus *Carnegiea gigantea* (Engelm.) Britton & Rose (Cactaceae, saguaro), provides food, shelter and other benefits to over 100 documented species (Drezner 2014). *Carnegiea* suffers widespread mortality in the colder portions of its range during periodic severe freezing events (Niering et al. 1963), which determine the northern edge of their range (Shreve 1911, Steenbergh and Lowe 1977, 1983). Columnar cactus morphology reflects climatic vari-

ations such as increases in diameter with winter rainfall (Drezner 2003a), and narrower stems where winters are relatively warm (Cornejo and Simpson 1997). Ribs are more closely spaced and furrows deeper on the south sides of *Carnegiea* plants compared to north sides (Yeaton et al. 1980). *Carnegiea* ranges further north than *Pachycereus pringlei* (S. Wats.) Britton & Rose, *Lophocereus schottii* (Engelm.) Britton & Rose, and *Stenocereus thurberi* (Engelm.) Buxb., and averages greater spine shading than these taxa (Geller and Nobel 1986). *Carnegiea gigantea* apical spine shading increases with latitude from about 9% at 30.5°N to about 41% at 35°N latitude (Nobel 1980b). Spines and pubescence protect meristematic tissue extending the range of *Carnegiea* and other columnar cacti northward (Nobel 1980a, b). Stem shading by spines increases with elevation for other cacti such as *Cylindropuntia acanthocarpa* (Engelm. & J. M. Bigelow) F.M. Knuth (Nobel and Bobich 2002). While variations in spine morphology may be genetic, the variability observed even over small distances (e.g., Yeaton et al. 1980) suggests that phenotypic plasticity in spine length likely reflects changes in local environmental conditions. The importance of such morphological adaptations will increase with climate warming and range readjustments.

The purpose of this study is to assess spine length at a given site across a variety of microenvironmental conditions, i. e., shade, proximity to competing individuals, presence near surface water (runnels) and north versus south sides of plants, to assess variation in spine length in two different populations. Differences in spine length associated with surface water, shade, orientation and proximity to conspecifics will be compared in a dry, more sparse

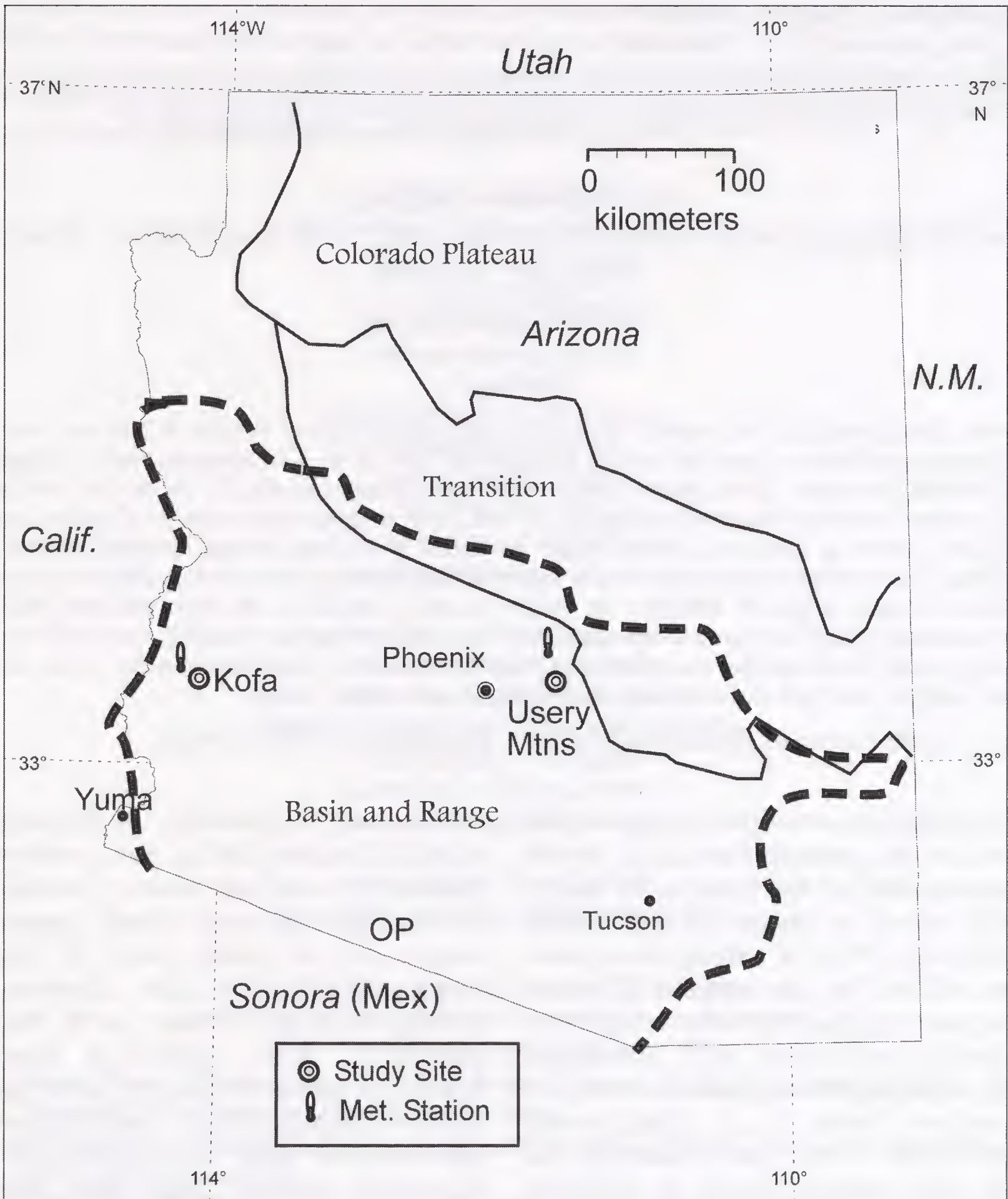


FIG. 1. Locations of the two study sites, Kofa and UM, along with the meteorological stations used (the Quartzsite station is about 16 km from Kofa, Bartlett Dam is about 37 km from the UM site). The dashed line represents *Carnegiea*'s entire United States range. The location of Organ Pipe Cactus National Monument is provided for reference (OP). Physiographic regions (Basin and Range, Transition, Colorado Plateau) from Rasmussen (2006, and references therein).

population in the west and in a wetter and more vegetatively lush population in the eastern Sonoran Desert to determine regional variation. Intraspecific competition for water and proximity to surface water channels are important as they represent the availability of water in a xeric ecosystem.

MATERIALS AND METHODS

Study Sites

The Kofa site (33.534°N, 114.165°W) is west of the western boundary of the Kofa National Wildlife Refuge at an elevation of 400 m above sea level, in the Lower Colorado River Valley subdivision of the Sonoran Desert (Shreve 1951, Turner and Brown 1994, Fig. 1). The Usery Mountains (UM, 33.487°N, 111.612°W, elevation about 650 m) are part of the

Arizona Upland subdivision of the Sonoran Desert (Shreve 1951, Turner and Brown 1994). The northern Sonoran Desert, where both populations are found, exhibits an east to west gradient in elevation (decreasing towards the Colorado River to the west), which is intertwined with a precipitation gradient (higher precipitation totals to the east) and a pH gradient (lower pH to the east) among other soil gradients over the region (Medeiros and Drezner 2012). Both sites are topographically flat (Kofa about 1.2%, UM about 3.0%; Drezner 2003a). Data for the Quartzsite meteorological station (#026865) near Kofa (Fig. 1) show that minimum temperatures in January average 2.7°C, while the Bartlett Dam meteorological station (#020632) near the UM site shows that minimum January temperatures average 4.6°C. By comparison, mean maximum July temperatures are 42.7°C near Kofa and 40.6°C near the UM site. The Sonoran Desert

TABLE 1. Statistical results and means. Sample size (n) for each t-test is given along with the mean length (mm) of the spines in that group (L), and the standard deviation (SD). The two sites are the Usery Mountains (UM) and Kofa sites. Other abbreviations include: north (N) and south (S) facing spines on the plant, and all north and south facing spines together (all spines). Other groupings include plants with other plants in close proximity (Clump), and those without nearby competitors for water (No Cl), high and low shaded plants, and plants in runnels, and not accessing runnels (No Run).

Site	Variable	n	L	SD	P
Between-site comparisons (t-tests)					
UM	S facing spines	51	67.8	11.8	0.74
Kofa	S facing spines	51	67.0	11.5	
UM	N facing spines	51	61.3	10.1	0.0495
Kofa	N facing spines	51	65.3	10.6	
UM	all spines	102	64.5	11.4	0.29
Kofa	all spines	102	66.2	11.0	
Within-site comparisons (paired t-tests)					
Kofa	N facing spines	51	65.3	10.6	0.19
Kofa	S facing spines	51	67.0	11.5	
UM	N facing spines	51	61.3	10.1	<0.001
UM	S facing spines	51	67.8	11.8	
Within-site comparisons (t-tests)					
Kofa	Clump	16	71.7	14.2	0.029
Kofa	No Cl	86	65.1	10.1	
UM	Clump	16	57.0	9.5	<0.01
UM	No Cl	86	65.9	11.2	
Kofa	High Shade	32	68.2	13.1	0.22
Kofa	Low Shade	70	65.3	10.0	
UM	High Shade	38	63.6	12.3	0.55
UM	Low Shade	64	65.0	10.9	
Kofa	Runnels	60	65.4	11.8	0.43
Kofa	No Run	42	67.2	9.8	
UM	Runnels	54	62.0	9.7	0.017
UM	No Run	48	67.4	12.6	

receives rainfall from extratropical cyclones in winter while monsoon-driven convective thunderstorms provide rainfall in summer (Carleton 1986, 1987, Sheppard et al. 2002). Mean January, July, and annual rainfall are 13 mm, 12 mm, and 128 mm, respectively in Kofa and 40 mm, 28 mm, and 341 mm, respectively, in the Usery Mountains. Thus, the climate in Kofa is cooler in winter, hotter in summer, and more arid than the Usery Mountains. The vegetation reflects these climate differences, with 7% vegetation cover at Kofa compared with 31% in the Usery Mountains (Drezner 2003b). There are 22 *Carnegiea* per ha at the Kofa site and 60 *Carnegiea* per ha at the UM site (Drezner 2006).

Field Methods

Collection was limited to plants between 2–4 m in height because spines shorten over time due to abrasion (Yeaton et al. 1980). Because spine shading decreases with stem height (Nobel 1980a), results would be biased and unreliable if the full height spectrum was used. At each site, collection began with the first arbitrary plant and continued to each *Carnegiea* individual encountered after ($n = 51$ at

each site). Following Yeaton et al. (1980), the longest (typically central) spine was measured on each plant at a height of 1.5 m on the north facing side and then on the south side. If the *Carnegiea* individual was in close proximity to at least one other cactus where some competition for water and/or root interaction would occur, a clump designation (=yes) was assigned to those plants. Assessment criteria included distance to the proximal cactus relative to plant height, as *Carnegiea* roots extend laterally away from the plant as far out as the plant is tall (Cannon 1911). Because of the natural clumped distribution of the species resulting from establishment around nurse plants, such identifications are typically clear. Because *Carnegiea* derive all their water from surficial sources (McAuliffe 1984, McAuliffe and Janzen 1986), plants were classified as in a runnel (area of concentrated surface runoff) thus gaining access to greater amounts of water, or on the intervening area (=no runnel). Degree of shading of the plant was assessed as little to no shade (low), and moderate to high (high) shade. This distinction between high and low shade was made in the field. Plants with large nurses or proximal vegetation blocking substantial light were classified as high shade.

Statistical Methods

The Shapiro-Wilk test was used to ascertain which variables deviated significantly from a normal distribution. Three t-tests were used to compare south-facing spines between the two sites, north-facing spines between the two sites, and all spines between the two sites (Table 1). Next, paired t-tests assessed any difference in length of north- versus south-facing spines at Kofa, and then repeated for the Usery Mountains. North and south aspect data were combined and t-tests were run to assess differences in spine length for clumped plants and plants not proximal to other potentially competing cacti (repeated for both sites). T-tests were run for low and high shade plants, and then for plants in and out of runnels at each site. The correlated Bonferroni technique was applied to the results to correct for type I errors associated with running multiple tests (Drezner and Drezner 2016).

RESULTS

Results of the Shapiro-Wilk normality tests showed that Kofa-north, Kofa-south, and UM-south did not deviate from normality at $P < 0.05$, and UM-north at $P < 0.01$. At the UM site, spines were significantly longer to the south (north: 61.3 mm, standard deviation [SD] of 10.1 mm, south: 67.8, SD 11.8, $P < 0.0001$). Clumped plants at UM had shorter spines (57.0, SD 9.5, unclumped 65.9, SD 11.2, $P = 0.0037$), and plants in runnels had shorter spines (62.0, SD 9.7 in runnels, 67.4, SD 12.6 outside of runnels, $P = 0.0169$, Table 1, Fig. 2). At Kofa, clumped plants had longer spines (71.7, SD 14.2)

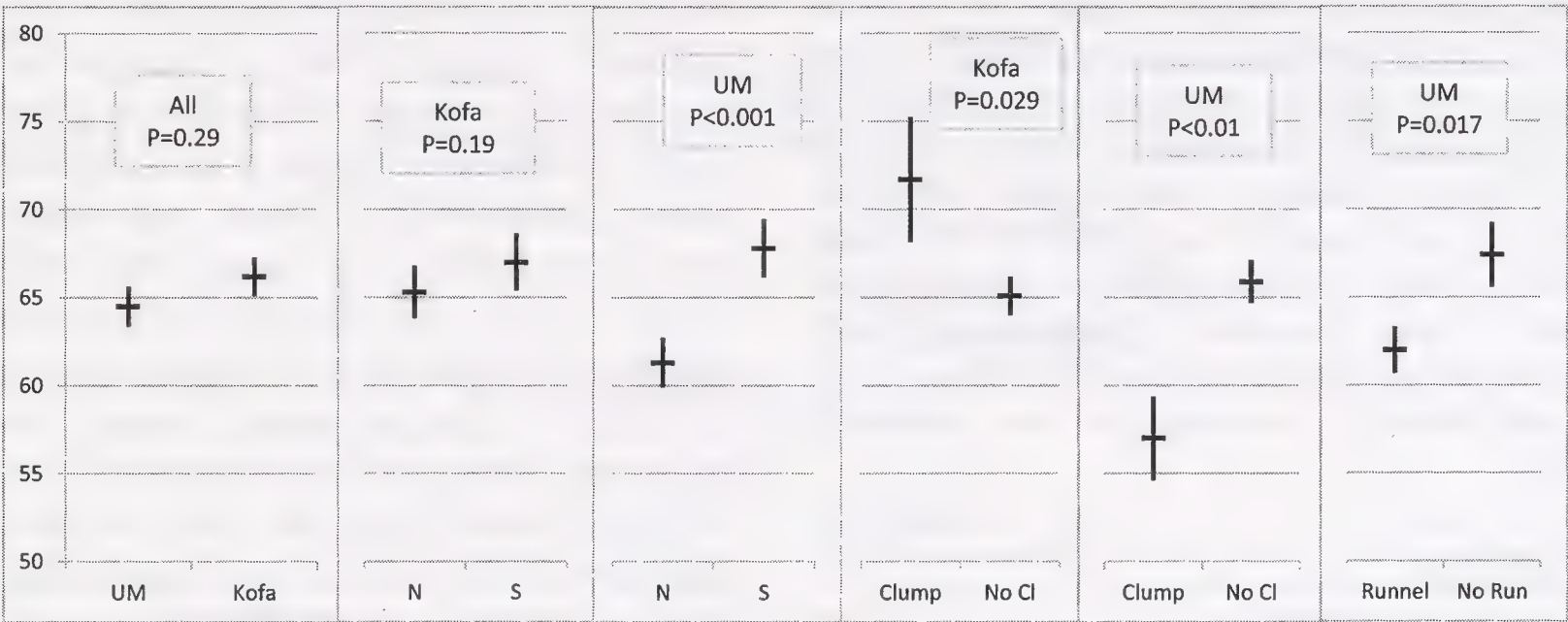


FIG. 2. Mean and standard errors of selected results including spine length (in mm on y-axis) comparing north (N) and south (S) sides of plants, plants in close proximity to other plants (Clump), and plants without potentially competing cacti nearby (No Cl), as well as comparing spine length on plants in and out of runnels (Runnel, No Run).

than unclumped plants (65.1, SD 10.1, $P = 0.029$), and north-facing spines at Kofa (65.3, SD 10.6) were longer than those at the UM site (61.3, SD 10.1, $P = 0.0495$). The correlated Bonferroni technique confirmed that all the results are statistically significant (Table 1).

DISCUSSION

Because *Carnegiea* apical spine shading increases to their northern range boundary, phenotypic plasticity in spine expression has been suggested (Nobel 1980b, Nobel 2002). Spines create a boundary layer around the plant, which moderates temperatures (Lewis and Nobel 1977, Drezner 2011). At the UM site, I found that south-facing spines were longer than north-facing spines (with the same trend at Kofa, though not significant). Plants also retain south, sun-facing spines more (Evans et al. 1994). Yeaton et al. (1980) found north-south differences in spine length only on slopes (versus topographically flat populations), which they attributed to greater water stress. However, *Carnegiea* can have faster growth rates on slopes than in topographically flat populations, which are associated with greater water uptake (Donnermeyer and Drezner 2012), so this assertion is variable.

Spine lengths are highly variable across and within populations. Compared to other published information, the variability in length observed here is confirmed. Average spine lengths (around 6.5 cm) are higher than the values reported by Yeaton et al. (1980) at Organ Pipe Cactus National Monument (5.76–6.29 cm averages; they list these values, presumably erroneously, as mm). Britton and Rose (1937, p. 164) identify spines as “often 7 cm long.” Variability in length may even be related to rainfall during the summer growing season or other factors influencing growth and health (Steenbergh and Lowe 1977).

Aridity, sunlight and high temperatures promote spine growth and coverage (Lewis and Nobel 1977, Yeaton et al. 1980, Nobel and Bobich 2002, Majure and Ervin 2008, Drezner 2011). Kofa is hotter in

summer, colder in winter, and drier than the UM site, and although not statistically significant, spines are longer at Kofa on average. South facing spines are similar at both sites, but north-facing spines are longer at Kofa (thus closing the N-S gap and yielding statistically non-significant results with comparable standard deviations) than at the UM site. At the UM, plants away from areas with concentrated drainage have longer spines, suggesting that longer spines are beneficial where less water is available. Thus, greater solar exposure promotes longer spines, as does reduced available surface moisture, following expectations of a species living in the low water, high summer temperature conditions found in the desert. The more extreme conditions may make spine length more consistent at Kofa than at the wetter UM site, where N facing spines and spines on plants in runnels are significantly shorter. The trend at Kofa is the same by direction and runnel access, but the difference is smaller and thus not statistically significant. Spines may be more important in the more extreme site, especially in otherwise less critical areas (N side, in runnels). Further, spine directionality differences would be less important on winter nights, providing protection in all directions in the colder Kofa. Cacti in colder sites often exhibit greater apical spine coverage (Nobel 1980c).

For clumping, results were significant at both sites but reversed. Clumping affects insolation receipt and water. Plants in close proximity cast shade, which is expected to reduce the need for spine coverage, but they increase competition for water (McAuliffe and Janzen 1986). At arid Kofa, plants in close proximity had longer spines, even with the increase in shade, a reflection of the aridity and relative marginality of this site. At the UM site, which receives nearly three times the amount of rainfall, the clumped plants (with increased shading) have shorter spines; the greater rainfall at the UM site appears adequate to compensate for the effects of intraspecific competition for water. Competition for water may reduce resource investment into spines (Nobel 1988) or alternatively, light limitation in a clumped setting reduces the need (or is disadvantageous) for spines

that also cast shade. Spines reduce photosynthesis in cacti (Nobel et al. 1991). Unclumped plants receive more insolation, and they compensate with longer spines.

The observed patterns in spine length suggest that where conditions are more extreme (more extreme seasonal temperatures, greater aridity), spine length increases, reducing photosynthesis and providing thermal protection. The more extreme site had more even spine lengths to the north and south (for example), seen primarily as longer spines in the direction away from the sun. At both sites, sun-facing spines were still longer, and generally large variability in spine length occurs within and across sites. Clumped plants that necessarily had increased competition for water but also greater shading were split; at the more extreme site, greater spine shading occurred where water competition was fiercest, while at the less extreme UM site, greater shading occurred in association with sunlight rather than water. The ability to adapt morphologically to changing conditions contributes to species success with changing environmental conditions. *Carnegiea* is likely to shift northward with warming climate (Rehfeldt et al. 2006) and the great variability in spine length, particularly in the two populations considered here which are towards the northern edge of the species' modern range, will be beneficial for *Carnegiea* as it adjusts to changing conditions. This includes range expansion into previously uninhabited areas as its range extends northward, as well as coping with increasing heat loads.

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A NEW SPECIES OF *APHYLLON* (OROBANCHACEAE) PARASITIC ON *GALIUM* IN THE WESTERN USA

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ABSTRACT

Aphyllon epigalium Colwell & A.C. Schneid. is described as a new species from Oregon and California. This taxon is distinguishable from the other members of *Aphyllon* Mitch. sect. *Aphyllon* in Western North America (i.e., *A. fasciculatum* [Nutt.] Torr. & A. Gray, *A. purpureum* [A. Heller] Holub, and *A. uniflorum* [L.] Torr. & A. Gray) in its host preference for *Galium* L., by having 2–4 yellow flowers per stem, and pedicels longer than the stem. Two subspecies are described: *Aphyllon epigalium* subsp. *epigalium*, which occurs in the Cascade and Klamath ranges in Oregon and California, and the Coast Ranges and Sierra Nevada in California; and *A. epigalium* subsp. *notocalifornicum* Colwell & A.C. Schneid., currently known from few sites in montane southern California. The new subspecies differ from one another in flower size, corolla lobe shape, host preference, geographic range, and nuclear and plastid genetic markers. An updated key to California and Oregon *Aphyllon* sect. *Aphyllon* is presented.

Key Words: *Aphyllon epigalium* subsp. *epigalium*, *Aphyllon epigalium* subsp. *notocalifornicum*, *Galium*, *Gymnocaulis*, *Orobanche*, Orobanchaceae, parasite, taxonomy.

For the past century, the concept of *Aphyllon* Mitch. sect. *Aphyllon* (formerly *Orobanche* L. sect. *Gymnocaulis* Nutt., see Schneider 2016) has included just two species, *A. uniflorum* (L.) A. Gray and *A. fasciculatum* (Nutt.) Torr. & A. Gray, both of which have large ranges in North America and occupy a variety of habitats and floristic regions. Previous efforts to segregate additional western North American taxa (Suksdorf 1900, 1906, Achey 1933) were not widely accepted. Recently, a combination of host-relationship, morphological, and molecular evidence has been used to distinguish the western North American populations of *A. uniflorum*, recognized under the name *A. purpureum* (A. Heller) Holub (Schneider 2016). Sufficient accumulation of herbarium specimens for analyses, coupled with phylogenetic evidence provided by Schneider et al. (2016) make it possible to convincingly parse this species complex into additional natural groups.

Within the California Floristic Province, both *A. purpureum* and *A. fasciculatum* display variability in coloration, size, number of flowers, and host preference. We here describe and name a distinctive entity in this region that has been noted informally as appearing intermediate between *A. purpureum* and *A. fasciculatum* by several earlier collectors. For example, in 1918, W. L. Jepson added the following annotation to his specimen 6385 (JEPS 10829), collected three years earlier and determined as *A.*

fasciculatum: “This has the habit of *O. uniflora* but the calyx segments are broad as in *O. fasciculata*. Moreover the color of the flowers is yellow as per note in Field Book. A specimen similar to this, Comptche, Harriet Walker 368, is in UC. Herb.” (Notes: Walker 368 is UC 112774; the entry in Jepson Field Notebook for 6385 can be found here: http://ucjeps.berkeley.edu/cgi-bin/display_fb.pl?page_no=31_89.) In 1956, G. Thomas Robbins put the following note on several specimens at CAS (Follette s.n., 1930, Howell 19834, Howell 21000): “In habit (particularly the reduced number of scapes) these plants suggest *O. uniflora*, but they have the broader calyx-lobes & the more pointed, non-ciliolate corolla-lobes of *O. fasciculata*.” Two decades later, in her master’s thesis on *Orobanche* sect. *Gymnocaulis* (Watson 1975), the second author of this paper described and proposed this entity as a subspecies of *Orobanche fasciculata* Nutt., but the name was not formally published.

Based on the distinct morphology and ecology of these plants, presented below, and with support from the molecular phylogenetic analyses previously cited, we conclude that plants parasitic on *Galium* L. in the western United States form a monophyletic group distinct from other previously described species, but sympatric with them. Furthermore, these *Galium* parasites warrant division into two subspecies

distinguishable by host preference, morphology, geographic range, and genetic distinctness.

TAXONOMIC TREATMENT

Aphyllon epigalium Colwell & A.C. Schneid., sp. nov. (Fig. 1) —TYPE: USA, California, Butte Co., Paradise, Elliot Rd, 39.7592 N, 121.5968 W, host *Galium bolanderi* A. Gray, 1 June 1974, K. D. Cox 188 (holotype: JEPS; isotypes: CHSC, SD).

Diagnosis: Distinct from all other species of *Aphyllon* in parasitizing *Galium* spp., and from other Western U.S. species of *A.* sect. *Aphyllon* in having a combination of both 2–4 yellow flowers per stem and pedicels longer than the stem (the combined vegetative and inflorescence axis).

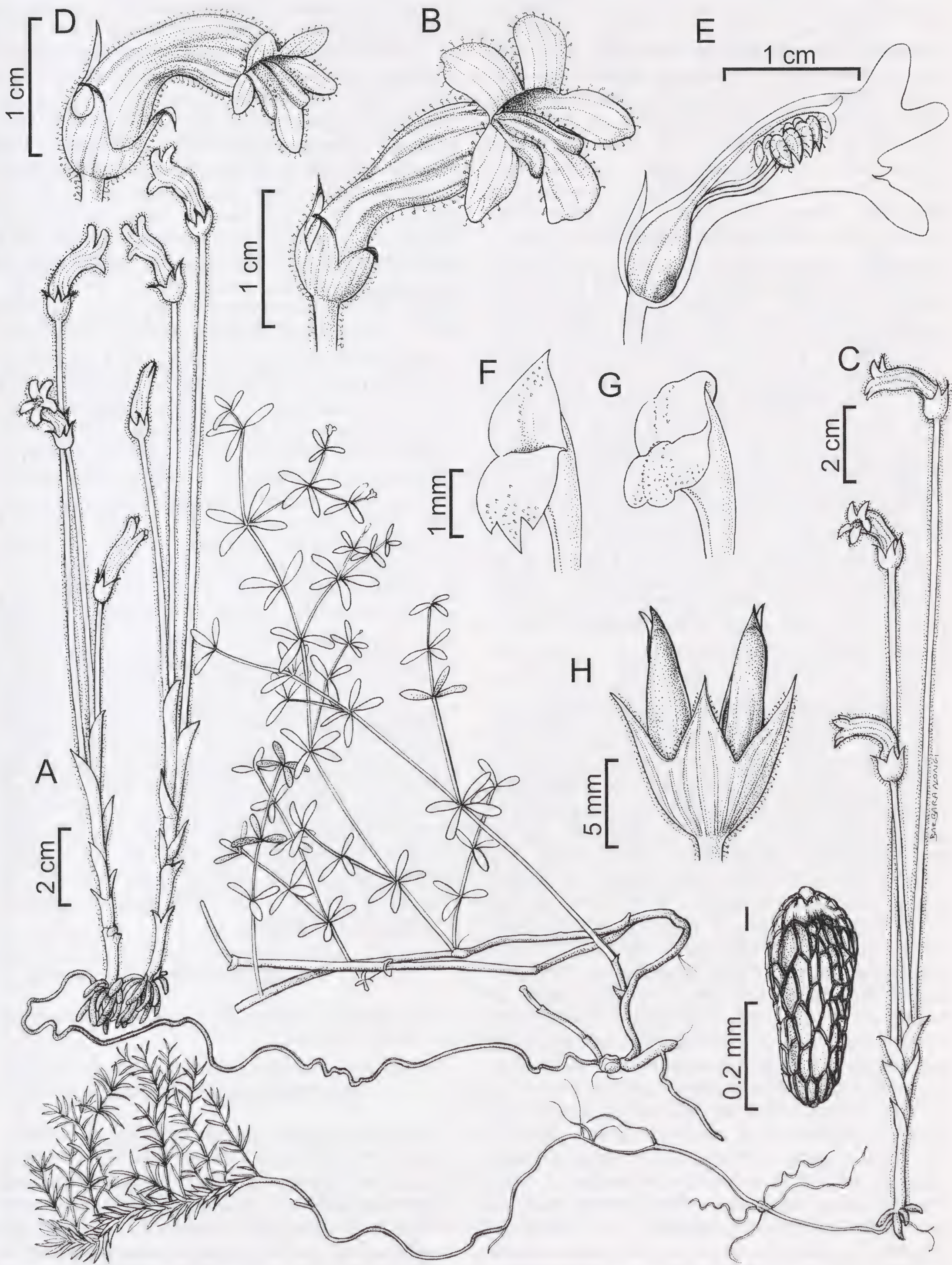
Plants annual or overwintering annual, 10–30 cm in length. **Roots** few to many, blunt, simple, succulent, up to 1 cm in length, often forming a mass around host attachment (primary haustorium), rarely with secondary haustoria. **Stem** unbranched or few-branched from basal host attachment point, succulent but slender and evenly thickened, 2–5 mm diameter. Stem length (from belowground host attachment point to terminus of inflorescence axis at ground level) 1–15 cm. Belowground structures glabrous, pallid; aboveground structures moderately to densely glandular-pubescent, straw-color to bright yellow, often tinged pinkish to purplish. **Leaves** few (these plus inflorescence bracts 2–10[–15] in number), reduced to scales 5–9 mm long, to 9 mm wide at broadest point, deltoid to linear, loosely imbricate or not overlapping, clasping stem, tips subacute to truncate, margins entire, glabrous. **Inflorescence** axis is a continuation of the vegetative stem, arising just below the soil surface. Flowers axillary, one per bract, (1–)2–4 per stem (in clustered or branched plants flowers appearing to be more numerous), the distal flower usually smaller than others, or aborted. **Bracts** 5–15 mm, intergrading with the reduced scale-leaves below inflorescence but trending larger, oblanceolate to obovate, loosely clasping, sometimes recurved at tip. **Pedicels** (6–)10–21 cm, erect; bracteoles 0. Proximal pedicels longer than the stem, distal pedicels sometimes not. **Calyces** (4–)5–11 mm, suberect, often purplish-tinged distally, sometimes entirely pinkish to reddish-purple, glandular-pubescent. Sepals fused at base into cup 3–7 mm deep, subequally divided distally into 5 narrowly triangular to subulate lobes 2–4 mm long, 1–3 mm wide. **Corollas** 13–30 mm, fused into a curved tube, constricted above ovary, then slightly inflated adaxially, narrowing at mouth, moderately glandular

pubescent; interior straw-colored to yellow, exterior similar, or pinkish, or tinged reddish, purplish, or finely purple-veined. Palatal folds evident, concolor or yellow, sparsely glandular-pubescent, inward-pouched between all but upper two lobes. Corolla lobes five, 2–4 mm long, 1–4 mm wide, similar in color to tube, oblong to obovate, erect to spreading and recurved at tip, more strongly so in age, tip margins rounded, truncate, minutely notched or apiculate, sparsely glandular pubescent. **Stamens** didynamous, with glabrous filaments; anther sacs villose-tomentose from longitudinal suture, glabrous on back, clustered below stigma. **Stigma** white, two-lobed, the lobes thin, triangular, usually with one or two subsidiary teeth, becoming recurved and thickened in age, included within corolla tube. **Ovary** two-chambered, placentation parietal, with orange-pigmented gland at abaxial base. **Capsules** 6–12 mm, ovoid to oblong-ovoid, dehiscent. **Seeds** 0.2–0.4 mm, brown, irregularly faceted, ovoid to oblong, most 2–3× longer than wide, surface favose.

Aphyllon epigalium* subsp. *epigalium Corolla yellow, often with reddish or purplish tinge or purplish venation externally, (17–)20–30 mm long, tube moderately arched such that flowers are suberect, with mouth of open flower presented at 45°, corolla lobes less than twice as long as wide, spreading and/or recurved as the flower matures

Paratypes: USA, CALIFORNIA, **Butte Co.:** Hills, June 1879, *Austin s.n.* (NY); Little Chico Canyon, May 1896, *Bruce* 828 (MO); Marysville Buttes, Rocky Slope of East Butte, 16 May 1902, *Heller s.n.* (NY); Jonesville, rocky crest of ridge between Willow and Colby Creeks, host *Galium bolanderi*, 10 June 1931, *Copeland* 1557 (CAS, RSA); north fork of the Feather River above Camp Rogers, on trail to Buck's Lake, 22 May 1957, *Balls* 22511 (RSA); Doon Grade, near Paradise, 19 May 1970, *Ahart* 55 (CHSC); Big Butte Creek, 11 May 1972, *Salinas* 2 (CHSC); Bluffs on E side of Humbug Rd., 2 mi NE of Centerville, 18 April 1980, *M.S. Taylor & Jokerst* 2539 (CHSC); Chino Ridge, host *Galium bolanderi*, 29 May 1981, *M. S. Taylor* 3814 (JEPS); Potter Ravine, 2 1/2 mi NE of Oroville Dam, *Ahart & Jokerst* 4961 (CAS, CHSC); Lake Oroville Recreation Area, Dan Beebe Trail, host *Galium porrigens* Dempster var. *tenue* (Dempster) Dempster, 13 May 1991, *Oswald & Ahart* 4585 (CHSC, UC); Lime Saddle Memorial Park, host *Galium porrigens* var. *tenue*, 15 May 1991, *Oswald* 4596 (CHSC); 1.5 mi ENE of Feather Falls, *Castro et al.* 513 (CHSC); Jackson Ranch Ridge, 0.9 mi ENE of Feather Falls, 1 June 1995, *Janeway* 4765. **Contra Costa Co.:** Mt.

FIG. 1. Illustration of *Aphyllon epigalium*. A–B. *A. e.* subsp. *epigalium*. A. Habit and connection to its host, *Galium bolanderi*. B. Fully open flower. C–D. *A. e.* subsp. *notocalifornicum*. C. Habit and connection to its host, *Galium andrewsii*. D. Fully open flower. E. Longitudinal bisection of typical flower, showing arrangement of stigma, anther sacs, and gland at base of ovary. F. Oblique frontal view of typical stigma at early anthesis showing a toothed lower lobe. G. Oblique frontal view of typical stigma at late anthesis showing thickened, recurved lobes. H. Dehiscent capsule. I. Mature seed, showing surface



reticulation. Illustration by Barbara Alongi. Source material: Parasite in A–B drawn from *Oswald & Ahart* 4585 specimen (UC 1586604), host plant in A from *Powell* 2790 (JEPS 82506), parasite and host in C–D from *Bell* 159 specimen (RSA 598503), E–G from a composite of *Mason* 12308 specimen (UC 1179027) and live material from *Schneider* 1029 and 1033 (JEPS, SBBG), H & I from *Jepson*, *s.n.*, Sisson specimen (JEPS 10831).

Diablo, Russelmann Park, host *Galium nuttallii* A. Gray, 16 May 1935, *Bowerman* 2995 (UC). **El Dorado Co.:** Placerville, 3 May 1941, *Reed s.n.* (UC); Placerville, 30 April 1943, *Robbins* 1050 (UC); 3 mi east of Placerville, host *Galium nuttallii*, 24 May 1944, *Robbins* 1632 (CAS, UC). **Humboldt Co.:** Jarinyan's, 12 July 1888, *Chestnut & Drew s.n.* (UC); Brannan Mountain, ridge north of Willow Creek, 7 July 1911, *Tracy* 3422 (UC). **Lake Co.:** April 1930, *Follette s.n.* (CAS); Eel River watershed, 21 June 1932, *Benson* 3725 (RSA); Four miles above Lucerne, host *Galium californicum* Hook. & Arn., 2 May 1936, *Mason* 11099 (GH, RSA, UC); Bartlett Mountain, 22 May 1940, *Mason* 12308 (UC, WS); Bartlett Mountain, Sawmill Flat, host *Galium californicum*, 18 June 1945, *Howell* 21000 (CAS, MO). **Madera Co.:** Upper Mariposa Grove, Yosemite Park, host *Galium bolanderi*, 19 July 2010, *Colwell* 10-107 (YM). **Mariposa Co.:** Turtleback Dome access road, Yosemite Park, host *Galium porrigens*, 9 June 2007, *Colwell* 07-56 (UC, YM). **Mendocino Co.:** Near Comptche, 23–29 June 1906, *Walker* 368 (UC); Round Valley, May 1954, *Irwin s.n.* (CAS); Confluence of Elder Creek with the South Fork of Eel River, 23–24 June 1961, *Sharsmith* 4876 (UC); Montgomery Woods State Natural Reserve, along Orr Springs Road, host *Galium muricatum* W. Wight, 28 May 2007, *Warner* 86 (JEPS). **Monterey Co.:** Monterey, *s.d.*, *Abbott s.n.* (CAS). **Napa Co.:** SR 37, 20 miles southwest of Monticello Dam, 12 May 1961, *Wardell* 27 (ARIZ, WTU). **Nevada Co.:** Alpha Road 1/2 mile north of Skillman Flat on Highway 20, 10 June 1965, *True* 2036 (CAS). **Placer Co.:** 1 mile west of Baxter, 7 May 1940, *Hitchcock* 6349 (WTU). **Plumas Co.:** Indian Valley, 1872, *Ames s.n.* (NY); *no locality, s.d.*, *Austin s.n.* (E); Greenville, 1 July 1924, *Stephens* 8329 (SD); Ridge NE of Humbug Valley, 7 June 1951, *Quick* 51-97 (CAS); Greenhorn Creek, 24 May 2004, *Ahart* 10948 (CHSC, JEPS). **Shasta Co.:** Castle Crag Trail, June 1893, *Dudley s.n.* (CAS); Burney, host *Galium bolanderi*, 30 May 1923, *Bettie s.n.* (CAS); 25 miles east of Redding, 20 May 1940, *Hitchcock* 6493 (WTU); East of Burney, 23 May 1941, *Cantelow s.n.* (CAS); Whiskeytown Lake, 29 May 1971, *Ganley* 640 (RSA); 2 miles south of Sweetbrier, 10 June 1980, *M. S. Taylor* 2816 (CHSC, JEPS); Clear Creek, 2.5 mi WSW of Clear Creek Rd and business I-5, host *Galium porrigens* var. *tenue*, 6 May 1981, *Powell* 2790 (JEPS); Hwy 299, 9 mi N of Redding, 9 May 1993, *Wisura* 4886 (RSA); Hamp Creek, 0.3 mi W of Ponderosa Way, 25 May 1993, *D. W. Taylor* 13501 (JEPS, RSA); Shasta Lake, Clikapudi Trail, host *Galium bolanderi*, 6 May 1994, *Oswald & Ahart* 6163 (CHSC, JEPS). **Siskiyou Co.:** Upper Sacramento, Hillside near Sisson, 24 July–10 Aug 1894, *Jepson s.n.* (UC); Black Butte, along Hwy 99, south of Weed, 22 June 1958, *Chisaki & Newcomb* 1154 (NY, UC, WS, WTU); Vicinity of Beaver Creek Basin, Siskiyou National Forest, 12 July 1944, *Dunkle* 9086 (RSA). **Sonoma Co.:** Armstrong Woods Park, 17 May 1976, *Harrison s.n.*

(JEPS). **Tehama Co.:** Between Mineral and Paynes Creek, 26 May 1927, *Grinnell s.n.* (JEPS); Log Spring Ridge, 9 July 1941, *Eastwood & Howell* 9730 (CAS); W of Paskenta near 15 mile post, host *Galium ambiguum*, 21 June 1953, *Baker & Wagnon* 12775 (RSA, UC). Brokeoff Mountain Trail, Lassen Volcanic National Park, host *Galium grayanum* Ehrend., 4 August 1986, *Oswald* 2267 (CHSC); Ides Cove Loop Trail, Long Lake, host *Galium ambiguum* W. Wight, 12 July 1988, *Oswald* 3552 (CHSC); Plum Creek Rd. SE of the town of Paynes Creek, host *Galium porrigens* var. *tenue*, 14 May 1997, *Oswald & Ahart* 8415 (CHSC); Off Hwy 32, 2 miles west of campground on Deer Creek, *Fischer* 835 (CHSC). **Trinity Co.:** Musser Hill, 25 May 1914, *Yate* 354 (RSA); North Yolla Bolly Mountains, Grasshopper Camp, 17 July 1951, *Munz* 16599 (RSA); 1 air mile SW of Mt. Eddy, Middle Deadfall Lake, 1 Aug 1971, *Heckard & Rubtsoff* 2696 (JEPS). **Tuolumne Co.:** South fork Stanislaus River, Italian Bar, 5 June 1915, *Jepson* 6385 (JEPS); Sonora, five miles east on Hwy 120, 19 May 1957, *Dodge* 172 (ARIZ), Big Oak Flat Road at lower end of Tuolumne Grove, Yosemite Park, host *Galium bolanderi*, 8 July 2004, *Colwell & Coulter* 04-83 (YM). **OREGON, Curry Co.:** Rogue River, near Hick's Creek, 21 June 1917, *Peck* 7239 (CAS, OSC); Mule Creek, 4 June 1928, *Leach* 1764 (OSC); Paradise Bar, 4 June 1917, *Nelson* 1367 (GH); W of Carpenterville, host *Galium* sp., 5 June 1929, *Henderson* 10157 (OSC); Bear Camp Pasture, 15 July 1978, *Stansell s.n.* (OSC). **Douglas Co.:** Rattlesnake Mountain, Umpqua National Forest, host *Galium oreganum* Britton, 27 July 2007, *Harvey s.n.* (OSC); SE of Steamboat, Umpqua National Forest, 14 July 2008, *Carr* 1559 (OSC). **Jackson Co.:** Sykes Creek, 26 May 1892, *Hammond* 320 (MO). **Josephine Co.:** Coast Mountains, Fortieth Parallel, 8 June 1884, *Howell* 242 (OSC). **Lane Co.:** H. J. Andrews Experimental Forest, host *Galium oreganum*, 16 Aug 1979, *Fitz* 1731 (HJAEF, scan of specimen reviewed for this study).

ETYMOLOGY

This species is named for its parasitic association with *Galium* species.

DISTRIBUTION AND ECOLOGY

Aphyllon epigalium subsp. *epigalium* is found in isolated populations over a large area, from southern Lake County, Oregon, to Madera and Contra Costa counties in California, with a single early record from Monterey County, CA (*Abbott s.n.*) (Fig. 3). This range includes sites in several ecoregions (Griffith et al. 2016), the Cascades and Klamath Mountains of both Oregon and California, the Coast Range, Central California Foothills and Coastal Mountains, Sierra Nevada, and Central California Valley (Sutter Buttes) in California.

Aphyllon epigalium subsp. *epigalium* flowers from late April to July, occasionally in August at higher elevations and higher latitudes. It occurs in light shade of open forest understory or brushy slopes in oak woodland, yellow pine forest, mixed conifer forest, red fir forest, and (rarely) exposed subalpine slopes (Fig. 2H). It has been found over an elevation range of 150–2650 m in foothill to montane zones on a variety of substrates, including igneous, metamorphic and serpentine, typically in the presence of a substantial duff layer.

Reported and observed host plants for this subspecies include *Galium ambiguum*, *G. bolanderi*, *G. californicum*, *G. grayanum*, *G. muricatum*, *G. nuttallii*, *G. oreganum*, *G. porrigens* var. *tenuis*, and *G. sparsiflorum* W. Wight subsp. *sparsiflorum*.

***Aphyllon epigalium* subsp. *notocalifornicum* Colwell & A.C. Schneid. subsp. nov. (Fig. 1) — TYPE:** USA, California, Riverside Co., Cleveland National Forest, Agua Tibia Wilderness, NE face of Eagle Crag, SE of the Crosley Saddle, South of the Cutca Trail, host *Galium andrewsii*, 15 June 1995, *Darin Banks 689 & Steve Boyd* (holotype: RSA; isotype: SD).

Diagnosis: *Aphyllon epigalium* subsp. *notocalifornicum* is distinct from *A. epigalium* subsp. *epigalium* in its paler, straw-colored flowers, often with a pinkish cast externally, its shorter corolla length of 13–20(–25) mm, its tube more prominently arched, with the corolla mouth presented \pm horizontally, its narrower corolla lobes (length $2\times$ width) that are either erect or weakly recurved at tips.

Paratypes: USA, CALIFORNIA. **San Bernardino Co.:** Mill Creek, June, *s.d.*, *Smith s.n.* (UC); **San Diego Co.:** Public Camp south of Cuyamaca Lake, 15 June 1937, *Roos 1466* (POM, UCR); Palomar Mountain, Fry Creek Campground, 29 June 1995, *Bell & Clemons 159* (RSA, SD); Agua Tibia Mountains, NE Face of Eagle Crag, 22 June 2016, *Schneider 1029* (topotype, JEPS). **Ventura Co.:** Divide Peak, Ocean View Trail, 16 June and 2 July 1948, *Pollard s.n.*, (CAS, RSA); Santa Ynez Mountains, Ocean View Trail near Divide Peak, 24 June 2016, *Schneider 1033* (JEPS, SBBG).

ETYMOLOGY

This subspecies is named for its distribution within the southern California Floristic Province.

DISTRIBUTION AND ECOLOGY

The known range of *Aphyllon epigalium* subsp. *notocalifornicum* is restricted to the Southern California Mountains ecoregion, specifically the Transverse and Peninsular Ranges in San Bernardino, Ventura and San Diego Counties.

Aphyllon epigalium subsp. *notocalifornicum* flowers from late May to early July. It occurs in light shade and open understory of slopes or riparian areas in oak woodland and conifer forest (e.g., *Pseudotsuga*

macrocarpa [Vasey] Mayr woodland); sometimes in patches of forest within chaparral (See Fig. 2G, I). It occurs over an elevation range of 1200–1500 m.

Specimens cited above report only a single species as the host, *Galium andrewsii* A. Gray, with the exception of two specimens examined (*Smith s.n.*, and *Pollard s.n.*), which do not mention a host.

DISCUSSION

Evolutionary Relationships

A recent molecular phylogenetic study of *Aphyllon* (Schneider et al. 2016) confirmed the existence of several strongly supported host-specific lineages in *A. sect. Aphyllon*. A clade comprising seven samples of *A. epigalium* was sister to a clade of *A. fasciculatum* parasitic on a variety of hosts, including *Eriogonum*, *Eriodictyon*, *Eriophyllum*, and *Phacelia* (Fig. 4), with an estimated divergence of 2 million years ago (A. C. Schneider, unpubl. data). A pair of samples representing the disjunct populations in southern California (= *A. epigalium* subsp. *notocalifornicum*) formed a strongly supported clade sister to the remaining five samples (= *A. epigalium* subsp. *epigalium*), providing additional evidence that the southern California populations are sufficiently distinct to merit recognition at the subspecific level.

Host Relationships

All confirmed hosts of *Aphyllon epigalium* belong to the genus *Galium*, and, with the exception of *G. oreganum* and *G. grayanum*, belong to a single clade of fleshy-fruited *Galium* found from Oregon to Baja California (clade 1 [*Galium* sect. *Baccogalium pro parte*] of Soza and Olmstead 2010). Strong host fidelity such as this is a widespread trait within the holoparasites of the Orobanchaceae, with some significant exceptions among weedy species of *Orobanche* and *Phelipanche* Pomel (Schneeweiss 2007). A large body of literature is devoted to the mechanisms of interaction between Orobanchaceae parasites and their hosts that result in host specificity or host shifts (Höniges et al 2012; Joel et al. 2013; reviewed in Fernández-Aparicio et al. 2016).

Host associations among species of *Aphyllon* have been long noted by taxonomists (Beck-Mannagetta 1890, Munz 1959, Heckard 1973), but recent treatments, benefiting from more extensive host assessments in the field, and more specimens with accurate ecological detail attached, have been able to define host associations more narrowly (Ellis et al. 1999, Collins et al. 2009, Colwell and Heckard 2012, Collins and Yatskievych 2015, Colwell and Yatskievych 2016). Molecular analyses have also highlighted the evolutionary importance of host specificity and corroborate the use of host identity in distinguishing morphologically similar taxa (Schneeweiss 2007, Thorogood et al. 2008, Schneider et al. 2016).

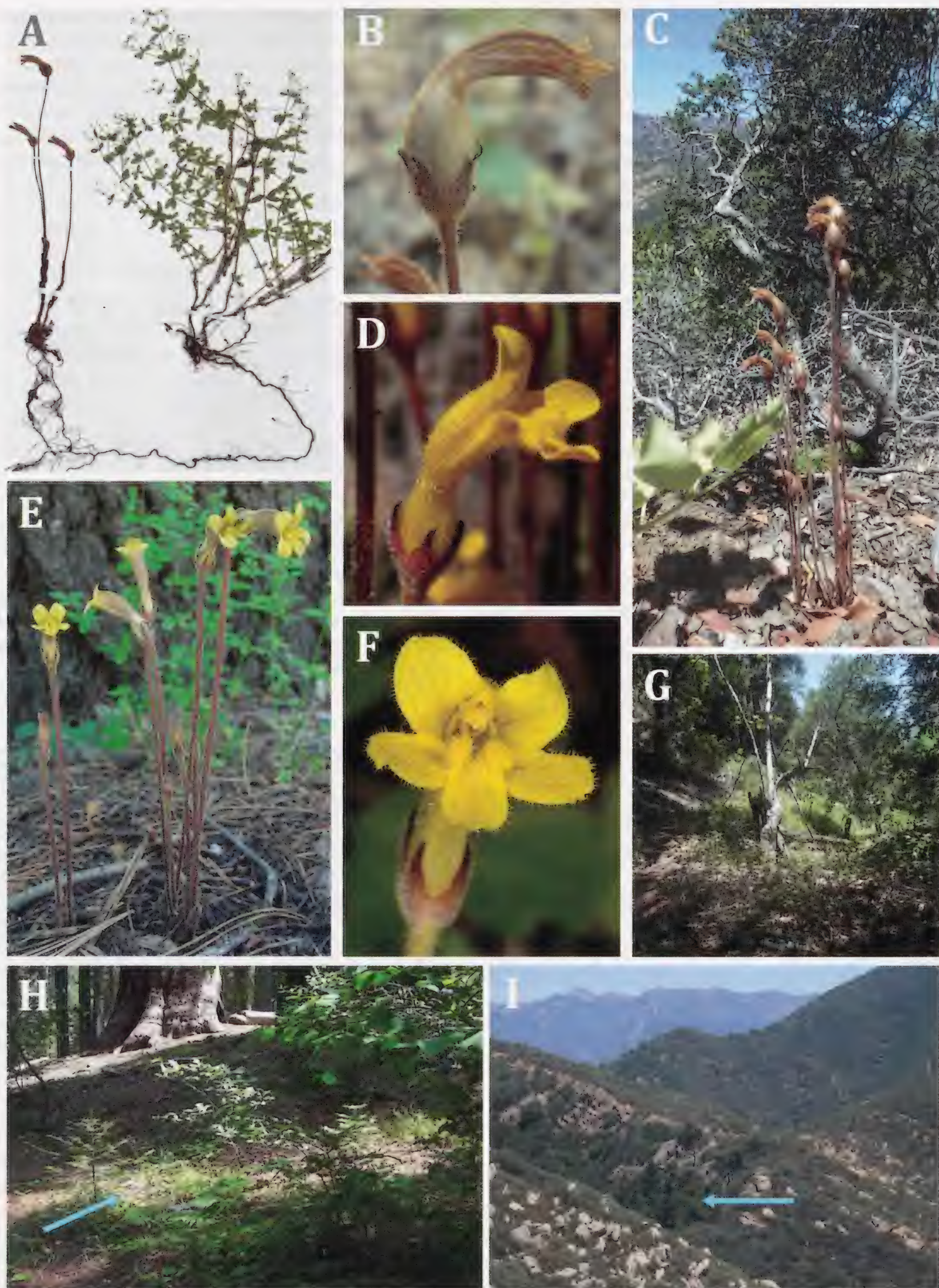


FIG. 2. Morphology and habitat of *Aphyllon epigalium*. *Aphyllon epigalium* subsp. *epigalium* (A, D–F, H). *Aphyllon epigalium* subsp. *notocalifornicum* (B–C, G, I). A. *Aphyllon epigalium* subsp. *epigalium* attached to a root of its host, *Galium sparsiflorum* subsp. *sparsiflorum* (Colwell & Coulter 04-83, YM). B. *Aphyllon epigalium* subsp. *notocalifornicum* flower at late anthesis, ovary beginning to swell (topotype, Schneider 1029, JEPS). C. Several plants of *Aphyllon epigalium* subsp. *notocalifornicum* at late anthesis/early fruit (Schneider 1033, JEPS, SBBG). D. *Aphyllon epigalium* subsp. *epigalium* flower at anthesis, lateral view (Siskiyou Field Institute, Josephine Co., OR. Photo courtesy Keir Morse). E. Several plants of

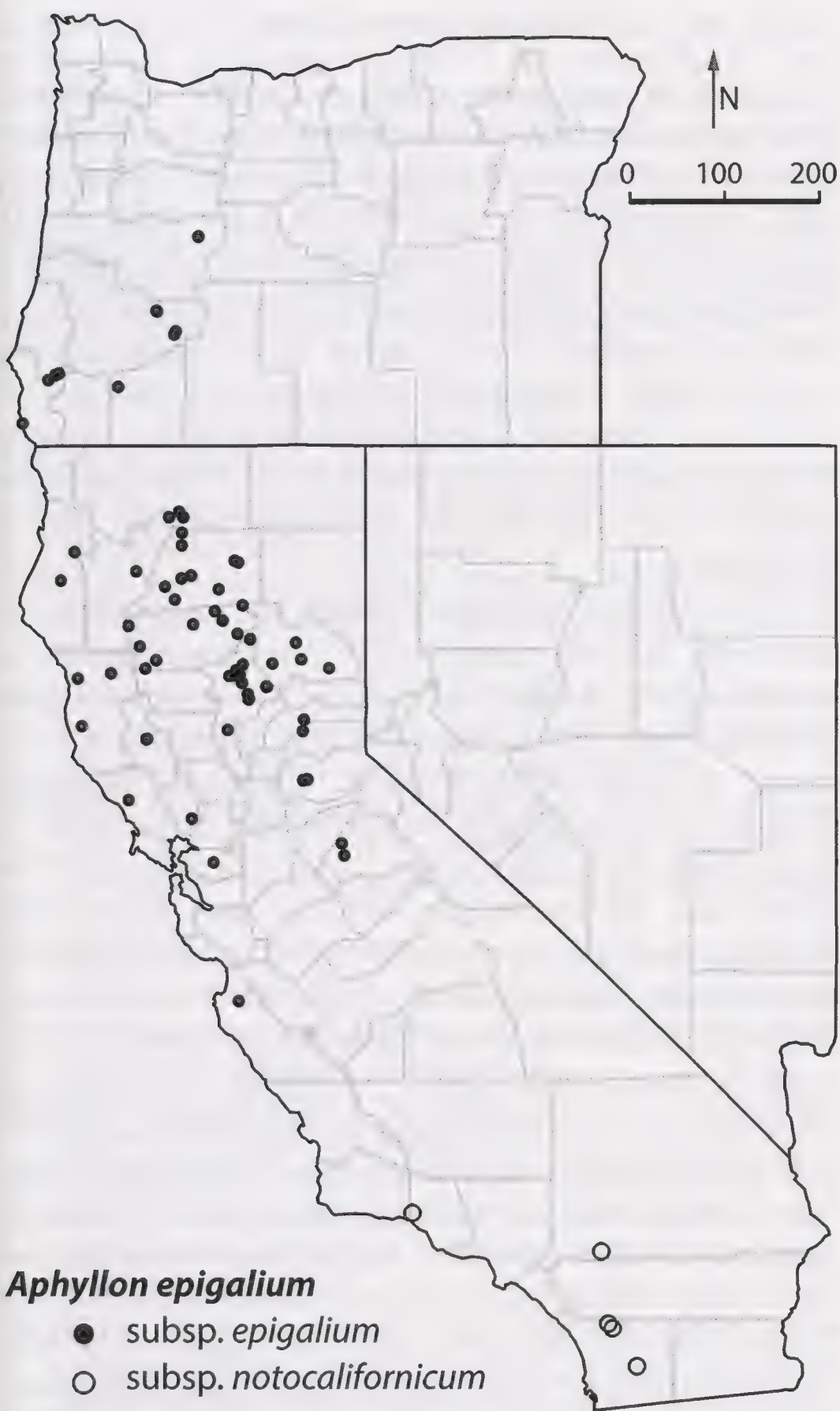


FIG. 3. Distribution of populations of *Aphyllon epigalium* in the western United States, with county boundaries shown in gray. Filled circles represent vouchered locations of *A. e.* subsp. *epigalium*. Open circles represent vouchered populations of *A. e.* subsp. *notocalifornicum*. Scale bar represents distance in kilometers.

Differing host associations between the two subspecies of *Aphyllon epigalium* appear to be consistent, even when the host of one subspecies is sympatric with the other parasite subspecies. For example, although the two subspecies are separated by a 330 km (200 mile) disjunction, from Merced County (the southern limit of subsp. *epigalium*), to the Santa Ynez Mountains in eastern Santa Barbara County (the northwestern limit of subsp. *notocalifornicum*) (See Fig. 3), some hosts overlap the range of both subspecies. Most strikingly, *Galium andrewsii*, the host of subsp. *notocalifornicum*, is abundant through-

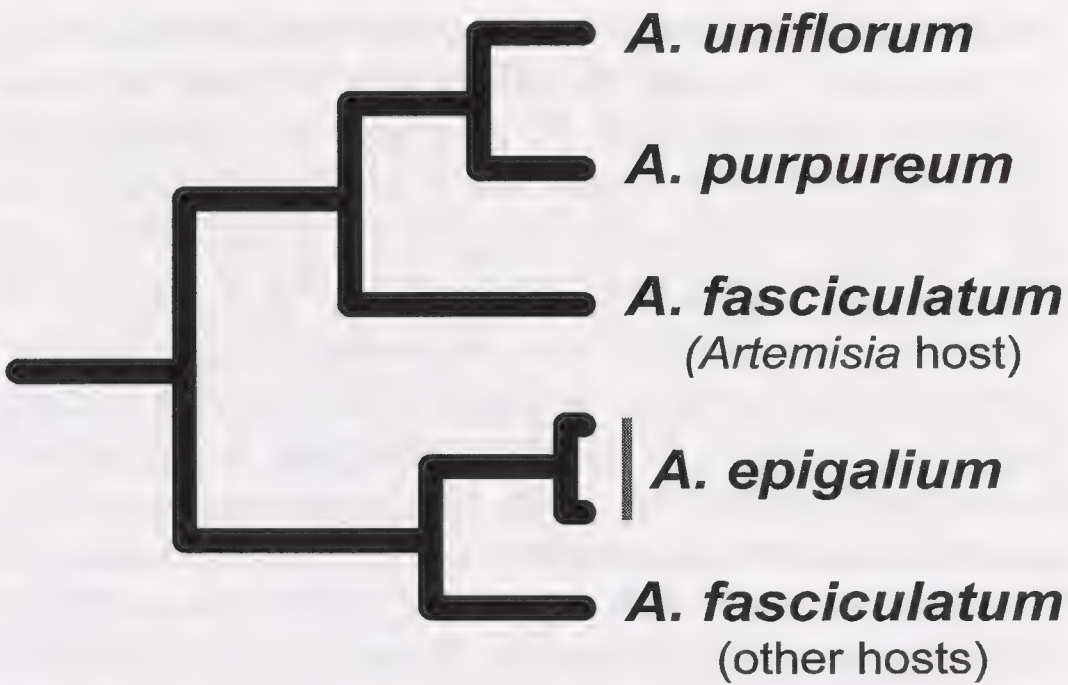


FIG. 4. Accepted phylogenetic relationships of all currently recognized taxa within *Aphyllon* sect. *Aphyllon*, including both subspecies of *A. epigalium*. All branches shown are strongly supported by ITS, ETS, and plastid DNA sequence data, posterior probability ≥ 0.97 . (Adapted from Schneider et al. 2016, Figs. 1–2).

out the inner Coast Ranges and has been collected as far north as 40° N, in Tehama County, CA. Although the distribution of this taxon overlaps considerably with that of subsp. *epigalium* throughout the North Coast Range, *G. andrewsii* is not a known host of *A. epigalium* subsp. *epigalium* even though it is very closely related to the other fleshy-fruited *Galium* species that are parasitized (Soza and Olmstead 2010). Similarly, we expect subsp. *notocalifornicum* to parasitize *G. californicum*, a host of subsp. *epigalium* that occurs within its southern California range, but no such association has yet been observed.

At even finer scales, differential host preference (or differential susceptibility on the part of host plants) may be occurring within populations. For example, in populations of *Galium*, certain individuals will be heavily infected by *Aphyllon epigalium*, while neighboring plants have no parasite load. In one population of *Galium bolanderi*, a dioecious host species, *A. epigalium* subsp. *epigalium* appeared to preferentially parasitize female host plants (A. E. L. Colwell, personal observation).

Abiotic Constraints

In regions where *A. epigalium* is present and a host is widespread, populations of *A. epigalium* nevertheless tend to be small and highly localized to pockets of unique plant communities within a larger matrix of conifer forest or chaparral (Fig. 2G, I). This suggests that other environment factors, such as host/habitat stability contribute to the scattered distribution of parasite occurrences. For example, fire regime may

Aphyllon epigalium subsp. *epigalium* with host (Yosemite National Park, Tuolumne Co., CA). F. Flower of *Aphyllon epigalium* subsp. *epigalium* at anthesis, frontal view (Carr 1559, OSC, photo courtesy of Gerald D. Carr). G. Type locality of *Aphyllon epigalium* subsp. *notocalifornicum*, Agua Tibia Wilderness, San Diego Co., CA. H. Habitat of *Aphyllon epigalium* subsp. *epigalium* in a *Sequoiadendron giganteum* (Lindl.) J. Buchholz grove. Blue arrow indicates collection site Colwell & Coulter 04-83 (YM). I. Habitat of *Aphyllon epigalium* subsp. *notocalifornicum* in a *Pseudotsuga macrocarpa* grove surrounded by chaparral, Santa Ynez Mountains, Ventura Co., CA. Blue arrow indicates collection site of Schneider 1033 (JEPS, SBBG).

enhance the distribution of *A. epigalium* either directly or mediated through its effects on its host in some habitats. Germination in response to exposure to smoke absorbed in water has been demonstrated for the related *Orobanche aegyptiaca* (Nun Bar and Mayer 2005). Similarly, charred wood is known to stimulate the germination of *Galium angustifolium*, which is closely related to known host species (Keeley and Keeley 1987). In the Sierra Nevada, low-intensity burns are associated with the regeneration of *G. sparsiflorum*, but more severe burns lead to declines in cover of this species (Knapp et al. 2007; Rocca 2009). In the central Sierra Nevada, flowering individuals of *A. epigalium* were observed by the first author to be more numerous following low intensity prescribed burns in the region's sequoia groves, although whether in this case it was due to host becoming more abundant, or to a direct germination response of the parasite to smoke is not known.

In other contexts, fire may limit the distribution of *A. epigalium*. A study of post-fire vegetation regeneration in the Santa Ynez Mountains found that *G. andrewsii* was sprouting in unburned areas, but not observed in burned areas during the two years post-fire (Christiansen and Muller 1975). It may be the case that this *Galium* species behaves as a fire-avoider in chaparral habitat. Thus, it is notable that the population of *A. epigalium* subsp. *notocalifornicum* from the Santa Ynez Mountains was not found in the chaparral, but in a small grove of *Pseudotsuga macrocarpa* surrounded by chaparral (Fig. 2I). This host-parasite combination may be utilizing a mesic microhabitat within the more widespread chaparral community of this region, and presence of the parasite could indicate a long history of stable host presence in this particular area. In either case, fire may play a key role in the robustness and distribution (e.g., patchiness) of parasite occurrences.

Conservation Status

Aphyllon epigalium subsp. *epigalium* is known from approximately 100 locations distributed widely across California and Oregon. However, the size of each population is small, typically fewer than twenty flowering individuals clustered around relatively few host plants, and thus each population is vulnerable to local extirpation. On the other hand, each capsule can produce thousands of seeds and studies of *Orobanche* indicate a long-term persistence of seed banks (Murdoch and Kebeab 2013). Therefore, we suggest that this species be evaluated for rarity regionally.

In contrast, *A. epigalium* subsp. *notocalifornicum* is currently known from only five populations in southwestern California. Two of these populations (Ocean View Trail, Santa Ynez Mountains, and along the trail to Eagle Peak, Cleveland National Forest) were confirmed extant by the last author in June 2016 (Schneider 1029, 1033; Fig. 2B, C). The third site, discovered in 1995 at the Fry Creek Campground on Palomar Mountain, was visited in 2016 but no individuals of *A. epigalium* were found. A fourth population was recorded in the early 20th century from Mill Creek, probably in vicinity of Mentone in the San Bernardino Mountains. The fifth and southernmost population near Cuyamaca Lake, San Diego County, was last collected in 1937. Given the wide distribution of its only known host, *Galium andrewsii*, within California and south to Sierra San Pedro Martír, Mexico, and the large distances between these five recorded populations, additional, undiscovered populations of *A. epigalium* subsp. *notocalifornicum* are expected to exist. Nevertheless, we recommend that this subspecies be considered for rare plant ranking.

KEY TO APHYLLON SECT. APHYLLON
IN WESTERN NORTH AMERICA

1. Flowers (3–)6–20 per stem; distal pedicels typically shorter than the stem (the combined vegetative and inflorescence axis); proximal pedicels as long as or rarely somewhat longer than the stem; hosts *Artemisia* L., *Eriodictyon* Benth., *Eriogonum* Michx., *Eriophyllum* Lag., *Phacelia* Juss. *A. fasciculatum*
- 1'. Flowers 1–4 per stem; distal pedicels as long or longer than the stem; proximal pedicels much longer (usually 2–3×) than the stem; hosts not as above
2. Flowers 1–2 per stem; corollas white to purple; corolla lobe margins ciliolate; palatal folds sometimes yellow; calyx lobes attenuate-filiform; hosts *Sedum* spp., Saxifragaceae, Apiaceae, Asteraceae
. *A. purpureum* (*Orobanche uniflora* L. subsp. *occidentalis* [Greene] Abrams ex Ferris)
- 2'. Flowers (1–)2–4 per stem; corollas cream to yellow, sometimes tinged pink or purple; corolla lobe margins glandular hairy; palatal folds concolor; calyx lobes narrowly triangular; hosts *Galium* spp. *A. epigalium*
3. Corollas yellow, often tinged reddish or purplish externally, (17–)20–30 mm long; corolla lobes spreading, length to width ratio < 2; hosts *Galium* spp. (but not *G. andrewsii*); southern Oregon to central California *A. epigalium* subsp. *epigalium*
- 3'. Corollas straw-color with pink or lavender cast externally, 13–20(–23) mm long; corolla lobes erect to slightly recurved at tip, length to width ratio ≥2; host *Galium andrewsii*; southern California.
. *A. epigalium* subsp. *notocalifornicum*

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CORRECTION TO “LATE SUMMER FOG USE IN THE DROUGHT DECIDUOUS SHRUB, *ARTEMISIA CALIFORNICA* (ASTERACEAE)”

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Due to a computational error while correcting plant water isotope samples for soil water evaporative fractionation, the results presented in Figures 2 and 3 are incorrect.

After correcting the computational error, an improved analysis of the plant water isotopes was conducted using a MixSIAR Bayesian mixing model v3.1 in R (Stock and Semmens 2013). The Markov Chain Monte Carlo chain length was 100,000 generations with a burn-in of 50,000 generations. This is a more robust method to determine proportional water use in plants than the method used in the original paper (Phillips and Gregg 2001).

Following the computational correction and improved data analysis, the isotope results in Figures 2 and 3 have changed. Plant water isotopes are now more depleted in the hydrogen and oxygen isotope than the water sources (corrected Fig. 2). It now appears that *Artemisia californica* Less. does not rely on fog water in the late summer months (corrected Fig. 3).

While the isotopic results imply no direct uptake of fog, the increased water content in *A. californica* for October 2011 (Fig. 4, not shown), suggests that fog may reduce evapotranspiration allowing shrubs to rehydrate from groundwater sources in the late

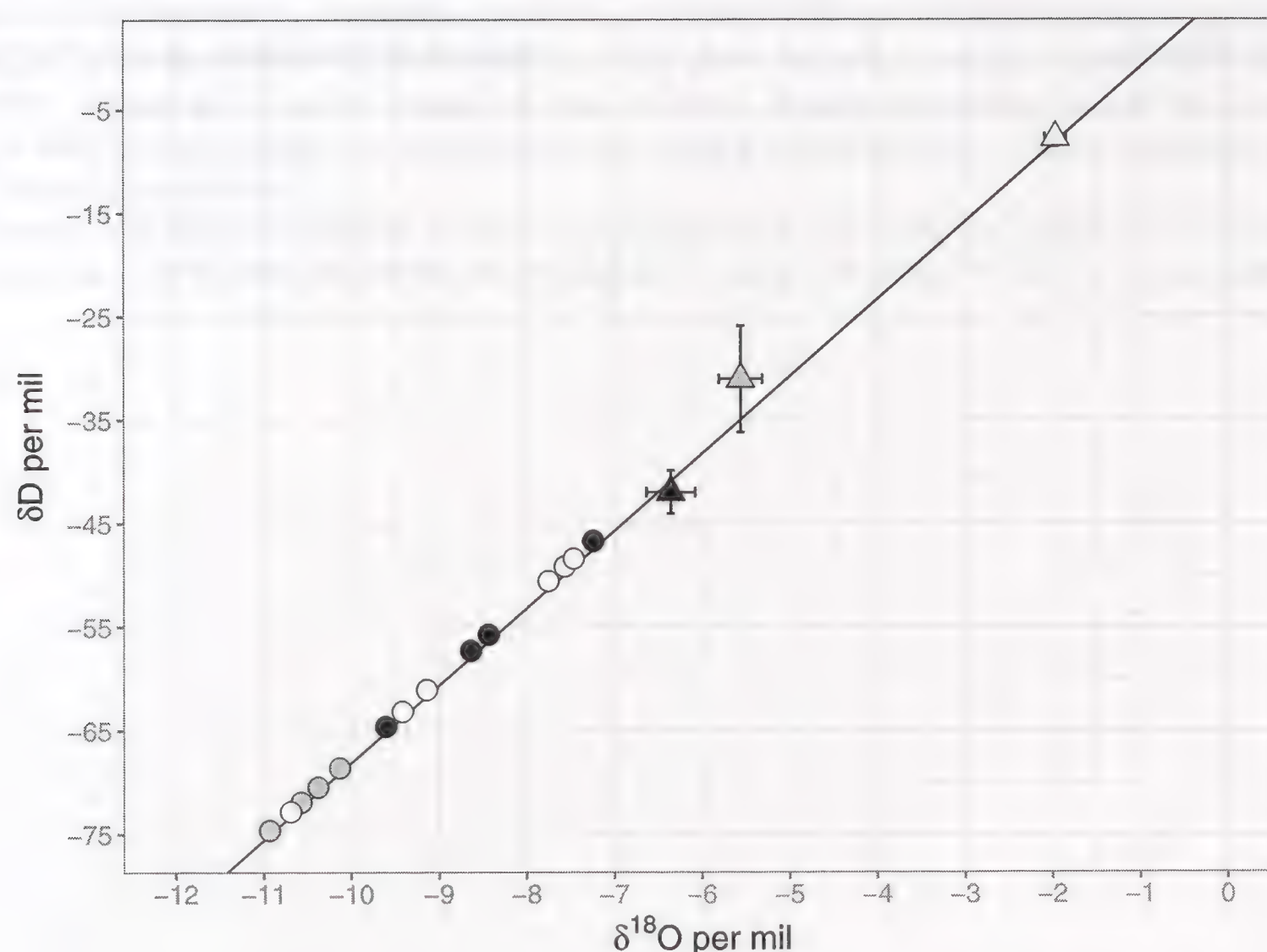


FIG. 2. The isotopic signatures of water and *Artemisia californica* stem tissue at Coal Oil Point Reserve. Fog is isotopically enriched in the hydrogen and oxygen isotopes (white triangle). Rain and groundwater are depleted (gray and black triangle, respectively). Plant water samples from July (black circles), August (gray circles), and September (white circles) were corrected to the local meteoric water line (black line). Error bars are the standard error associated with source water isotopic values.

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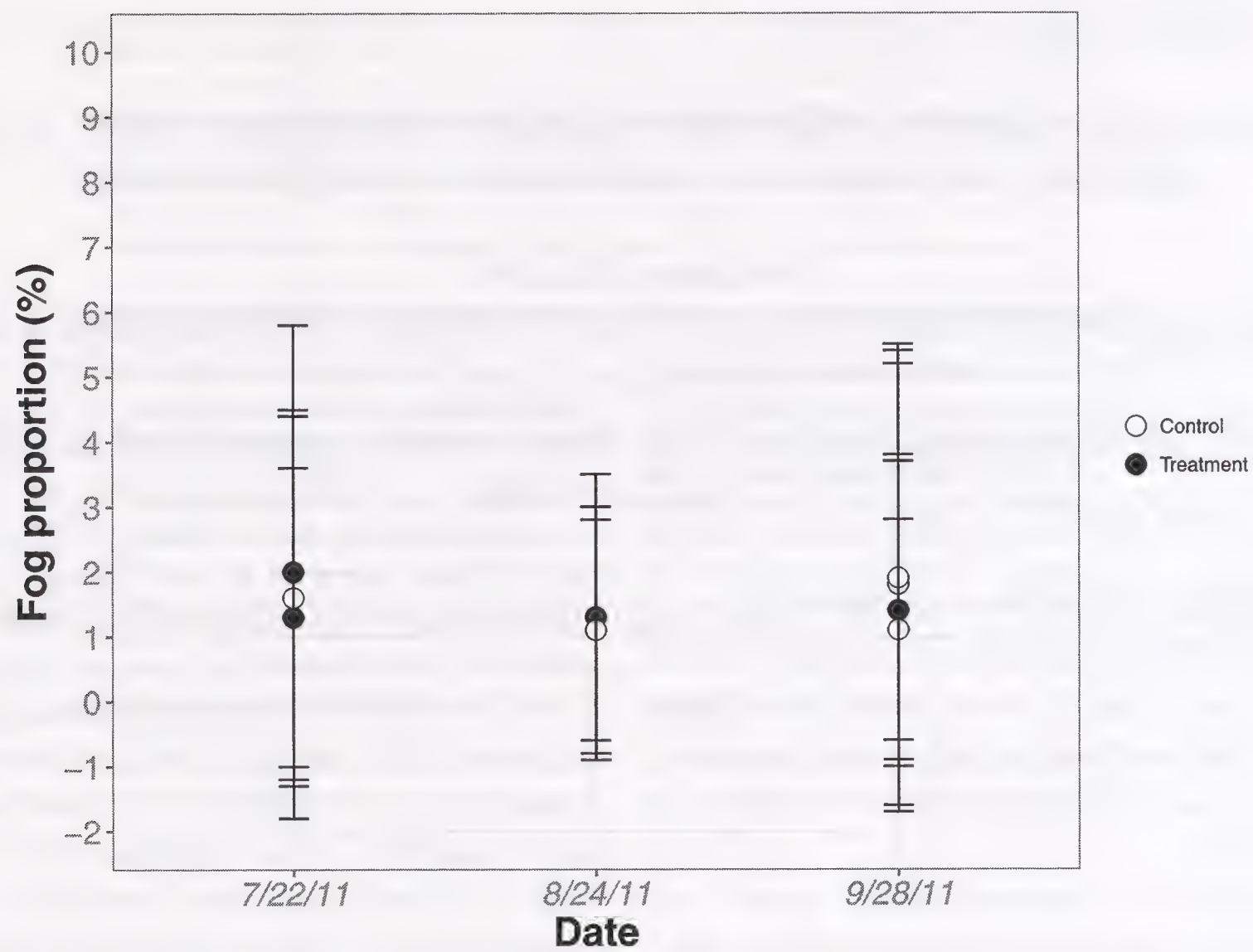


FIG. 3. Hierarchical Bayesian mixing model results for fog proportion in stem water for 2011. Each point is an individual plant (white for control, black for treatment) with the mean and standard deviation of fog proportion according to the model.

summer. However, it is possible that *A. californica* can take in fog water through their leaves, but by sampling stem tissue infrequently, evidence of this process remains lacking. Overall, this study suggests that fog effects on *A. californica* are likely temporary and could ameliorate summer drought conditions in coastal Southern California.

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SCHIZACHYRIUM SCOPARIUM (Michx.) Nash (POACEAE).—Monterey County, Gigling Road, on the former Fort Ord, elev. 99 m, 36.643795, -121.77535153315428, 6 November 2007, *D. Styer s.n.* (UCSC); the same location, 14 October 2015 *D. Styer & J. Styer s.n.* (UCSC); same location, 10 Nov 2008, *D. Styer s.n.* (PGM, specimen processed by Vern Yadon, and identification entered as var. *scoparium*, following the treatment in FNANM [Wipff 2003]).

I also collected a specimen (*D. Styer s.n.*) that was sent to James P. Smith for HSC. Growing on road edge depression, along with other grasses (e.g., *Aira caryophyllea* L., *Avena barbata* Link, *Briza maxima* L., *Vulpia myuros* [L.] C. C. Gmel.), forbs, a few shrubs (e.g., *Acmispon glaber* [Vogel] Brouillet var. *glaber*, *Artemisia californica* Less., *Baccharis pilularis* DC. subsp. *consanguinea* [DC.] C. B. Wolf, *Salvia mellifera* Greene, and *Toxicodendron diversilobum* [Torr. & A. Gray] Greene), and an oak (*Quercus agrifolia* Née var. *agrifolia*) nearby.

Previous knowledge. The only other California collection of *S. scoparium* listed by the California Consortium of Herbaria was made by Beecher Crampton at a location in Solano County on 12 Oct 1965 (CCH 2017). Calflora (2017) lists an observation on 23 Mar 2008 by David Magney in the Spenceville Wildlife Area in Nevada County. The natural range of *Schizachyrium scoparium*, little bluestem, is widespread, extending from Alberta to eastern Canada and south to Florida and Texas (Gleason and Cronquist 1991), and is one of the famous grasses of the tall-grass prairies. An adaptation for dryer parts of the tall-grass prairies enables *S. scoparium* to take hold when escaped into rather dry locations, such as a Fort Ord roadside. This grass is sold in the nursery trade in California. Since Gigling Road has a history of machinery and vehicle traffic, *S. scoparium* could reasonably have arrived directly from its native habitat, or as a result of the nursery trade. *Schizachyrium scoparium* is not naturally adapted to California's Mediterranean climate, and it doesn't grow well in the dryer years. Since most years are dryer than average, there is no indication that this species will become invasive in California.

Significance. The Gigling Road site may be the first evidence that a roadside population may have lasting endurance. At this point it has survived for at least ten years, even through the severe drought year of 2015, without any attention. Vern Yadon noted that this cespitose species could easily go unnoticed along California roadsides, especially because it grows less than a meter high, and has a very late blooming habit. The earliest record I have of an inflorescence is 21 August; this was well before bloom. Anthesis and fruit are to be expected from October on. Another reason that *S. scoparium* populations along roads could go unnoticed is danger. Gigling Road, at the *S. scoparium* location is, basically, open only to authorized vehicles; I comfortably stop there at my leisure. In contrast, I have one record of *S. scoparium* along Fort Ord's South Boundary Road. The cars go by at about 50 mph, and the barbwire fence is about 3 ft off the road. I'm aware that I don't go back because of the danger of being hit.

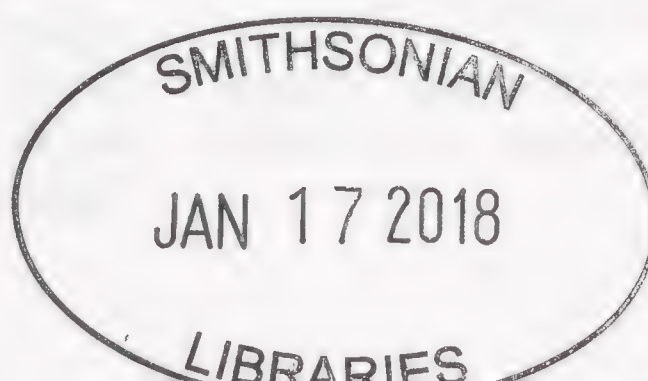
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NOTEWORTHY COLLECTIONS

MONTANA

ANELSONIA EURYCARPA (A. Gray) J.F. Macbr. & Payson (BRASSICACEAE).—Beaverhead County, Beaverhead Range, scattered colonies in limestone talus on a gentle, SE-facing slope of the ridge 1 km N of Deadman Pass with *Ericameria suffruticosa* (Nutt.) G. L. Nesom and *Minuartia nuttallii* (Pax.) Briq., 44.3776°N, 112.8337°W, 3110 m, 7 July 2015, *P. Lesica 11330 with J. Salix* (MONTU 162925).

Previous knowledge. Known from east-central Idaho and the Sierra Nevada Range of central California and adjacent Nevada (Kartesz 2015).

Significance. First record for Montana, a range extension ca. 25 km N from adjacent Lemhi Co., Idaho (CPNWH 2017).

CALTHA LEPTOSEPALA DC. var. *SULFUREA* C. L. Hitchc. (RANUNCULACEAE).—Beaverhead County, Beaverhead Mountains, Beaverhead-Deerlodge National Forest, seepage area in whitebark pine woodland on the northeast ridge of Eighteenmile Peak, ca. 30 km SW of Lima, with *Carex scopulorum* Holm, *Deschampsia cespitosa* (L.) P. Beauv., and *Veronica wormsjoldii* Roem. & Schult., 44.4508°N, 112.9877°W, 2955 m, 28 June 2006, *E. Evert 41704* (RM 913912); Italian Peaks, abundant in wet soil along a small stream at the head of Nicholia Creek with *Ranunculus eschscholtzii* Schldtl., 44.3678°N, 112.8469°W, 2865 m, 2 July 2014, *P. Lesica 11137* (MONTU 162237).

Previous knowledge. Known from Custer and Lemhi counties, Idaho (Hitchcock and Cronquist 1964).

Significance. First collections of this narrow endemic from Montana, ca. 40 km E of the nearest location in Lemhi Co., Idaho (CPNWH 2017). Although not recognized in the Flora of North America treatment (Ford 1997), this yellow-flowered, calciphilic variety occurs in an area of high endemism and is allopatric with typical var. *leptosepala* (Hitchcock and Cronquist 1964).

CAMPANULA PERSICIFOLIA L. (CAMPANULACEAE).—Lake County, along the side of Highway 35, 2 km of Yellow Bay, 47.8642°N, 114.0294°W with *Centaurea stoebe* L. and *Dactylis glomerata* L., 915 m, 25 July 2007, *P. Lesica 9762* (MONTU).

Previous knowledge. Native to Eurasia and North Africa, introduced primarily to northeastern and northwestern North America (Kartesz 2015).

Significance. First report for Montana, a range extension 250 km E from Bonner Co., Idaho (CPNWH 2017).

CREPIS NICAENSIS Balb. ex Pers. (ASTERACEAE).—Flathead County, scattered plants in a wet spruce forest ca. 2 km NE of Whitefish with *Carex disperma* Dewey and *Equisetum arvense* L., 48.4283°N, 114.3256°W, 930 m, 25 July 2012, *P. Lesica 10862* (MONTU 160159, OSC 243493). Determined by K. Chambers (OSC).

Previous knowledge. Native to Europe, sparingly introduced to northwestern and northeastern U.S. (Kartesz 2015).

Significance. First verified report for Montana, a range extension of ca. 600 km from Snohomish Co., Washington (CPNWH 2017).

DIPSACUS LACINIATUS L. (DIPSACACEAE).—Big Horn County, Crow Agency, low seep area behind the Catholic church on Makawasha Ave., just W of Hwy I-90, 45.6023°N 107.4652°W, 970 m, 2 August 2016, *J. Doyle and M. Hammond s.n.* (MONTU). Determined by M. Lavin (MONT).

Previous knowledge. Native to Europe, introduced to Oregon and California and sporadic from Colorado to northeastern U.S. and adjacent Canada, listed as a noxious weed in Oregon, Colorado, Minnesota, Iowa, and Missouri (Kartesz 2015).

Significance. First verified report for Montana (CPNWH 2017).

DIGITALIS GRANDIFLORA Mill. (PLANTAGINACEAE).—Flathead County, Glacier National Park, large colony along the Camas Road just N of the bridge over McDonald Creek, with *Bromus inermis* Leyss. and *Cornus sericea* L., 48.5267°N 113.9977°W, 960 m, 3 August 2016, *P. Lesica 11484* (MONTU 163655, KANU). Verified by K. Barringer.

Previous knowledge. Native to Europe, introduced to Ontario and northeastern U.S. as far west as northeastern Minnesota (USDA, NRCS 2017).

Significance. First report for Montana and western North America (Kartesz 2015). Plants occur near an area where visitors from across the continent park their cars to fish in McDonald Creek.

STACHYS BYZANTINA K. Koch (LAMIACEAE).—Flathead County, Kalispell, plants up to 25 cm high, rare in a highly disturbed riparian corridor adjacent to Bowser Creek with *Cirsium arvense* (L.) Scop., *C. vulgare* (Savi) Ten., *Elymus trachycaulus* (Link) Gould, and *Pascopyrum smithii* (Rydb.) Barkworth and D. R. Dewey, 48.1972°N 114.3424°W, 900 m, 4 August 2016, *R. L. Quire 001* (MONTU).

Previous knowledge. A cultivated ornamental native to Eurasia and sporadically escaped on the west coast and northeastern U.S. and adjacent Canada (Kartesz 2015).

Significance. First report for Montana and the Northern Rocky Mountain region (CPNWH 2017).

TANACETUM COCCINEUM (Willd.) Grierson (ASTERACEAE).—Park County, Absaroka Range, ca. two dozen plants flowering in an open Douglas-fir forest of Pine Creek Campground around site #23 with *Poa pratensis* L. and *Symphoricarpos occidentalis* Hook., 45.4995°N 110.5237°W, 2020 m, 28 June 2014, *P. Lesica* 11127 (MONTU 162798, WTU 406817).

Previous knowledge. Native to Asia and introduced as an ornamental, sparingly escaped in northeastern North America and Colorado (Kartesz 2015).

Significance. First report for Montana and the Northern Rocky Mountains (CPNWH 2017).

UTRICULARIA OCHROLEUCA R.W. Hartm. (LENTIBULARIACEAE).—Glacier County, 20 km S of Browning, local in areas of shallow water in a calcareous fen S of Willow Creek with *Trichophorum pumilum* (Vahl) Schinz & Thell. and *Utricularia vulgaris* L., 48.61915°N 112.77669°W, 1230 m, 30 Aug 2012, *P. Lesica* 10903 and *T. Luna* (MONTU 157820, NHA). Determined by G. Crow (NHA). Deer Lodge Co., 6 km E of Anaconda, local in shallow depressions of a fen S of Lost Creek with *Chara* spp. and *Triglochin palustris* L., 46.1710°N 112.8641°W, 1525 m, 29 June 2014, *P. Lesica* 11136 (MONTU 162267, MSC). Verified by G. Crow (MSC).

Previous knowledge. Interruptedly circumboreal south to California, Minnesota and New York, disjunct in northwestern Wyoming (Kartesz 2015).

Significance. First report for Montana, a range extension of ca. 240 km north from Yellowstone National Park and ca. 800 km from southern British Columbia (CPNWH 2017). A collection of this species (*Martin s.n.*, 4 Jul 1909 [RSA]) is reported for Yellowstone National Park in Montana; however this is unlikely because only a small portion of the Park is in Montana, and there was little access to this area 100 years ago (J. Whipple, personal communication). This species is often considered to be a hybrid between *Utricularia minor* L. and *U. intermedia* Hayne, and purportedly occurs where the ranges of both species overlap (Hultén 1962).

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POPULATION STRUCTURE AND SITE CHARACTERISTICS OF THE RARE SHASTA SNOW-WREATH (*NEVIUSIA CLIFTONII*)

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ABSTRACT

The Shasta snow-wreath (*Neviusia cliftonii* Shevock, Ertter & D.W. Taylor; Rosaceae) is a rare shrub, endemic to areas near Shasta Lake in the eastern Klamath Range of northern California, USA. Since discovery in 1992, the number of known populations has increased from 3 to 33. Shasta snow-wreath is a thicket-forming shrub, thought to reproduce primarily vegetatively, where individual stems (ramets) arise from the root system. We provide the first descriptions of demography and site characteristics using data collected in eight populations of Shasta snow-wreath. We established permanently-marked transects and recorded the number of ramets, individual stem heights, and the number of inflorescences in 2011, 2012, and 2013. We also characterized sites by tree canopy, shrub components, fuels, vegetation, and ground cover. Using the number of ramets recorded and the total population area, we estimated the number of ramets in each population ('ramet population size'). Ramet population size ranged from 716 to 18,641 (mean = 5467), and the average maximum stem height ranged from 50 to 159 cm (mean = 104 cm). Larger ramet population size and taller stems were both associated with less tree cover. The two largest ramet population sizes were found at the two highest elevations and most west-facing sites. The average number of inflorescences per stem over three years ranged from 0.08 to 4.91 (mean = 2.76) and showed an increase with elevation. How Shasta snow-wreath will respond to succession or disturbances is unknown, but the negative relationship of ramet population size and canopy cover indicates that fire may have been important for influencing the population size historically. Continued monitoring of the studied populations, and the addition of more populations to the monitoring program, would be useful for detecting demographic changes and for better understanding the factors that govern Shasta snow-wreath.

Key Words: demography, eastern Klamath Range, endemism, *Neviusia cliftonii*, population monitoring, rarity, Rosaceae, Shasta Lake, Shasta snow-wreath.

The Shasta snow-wreath (*Neviusia cliftonii* Shevock, Ertter & D.W. Taylor) is a rare shrub in the Rosaceae with a distribution limited to the vicinity of Shasta Lake in the eastern Klamath Ranges of northern California, USA (Taylor 1993; Lindstrand and Nelson 2006; Lindstrand et al. 2016; Fig. 1). Shasta snow-wreath was first discovered in 1992 along Cedar Creek east of Redding, CA, at the base of an exposed, north-facing limestone outcrop (Shevock et al. 1992; Taylor 1993). Because the first three Shasta snow-wreath populations were all found on limestone rock formations, the species was originally described as a limestone endemic (Lindstrand and Nelson 2005, 2006). However, exploration of the region has revealed that most populations (57%) are found on non-limestone substrates such as shale, mudstone, and greenstone (DeWoody et al. 2012). The only congener of Shasta snow-wreath, *Neviusia alabamensis* A. Gray, also has a highly restricted range and occurs in several disjunct populations in the southeastern United States (Long 1989).

It is possible that Shasta snow-wreath was at one time more widespread and has become progressively more restricted to its current, isolated range (DeVore et al. 2005). Fossil evidence of a closely related plant (*Neviusia dunthornei* DeVore, Moore, Pigg & Wehr) in a Pacific Northwest Eocene flora supports the hypothesis that the former range of the species may have stretched as far as British Columbia (DeVore et al. 2004; DeVore et al. 2005). The persistence of Shasta snow-wreath near Shasta Lake has been attributed to the lack of volcanic deposition, glaciation, and the combination of low elevations and high precipitation levels (Lindstrand and Nelson 2006). The number of known Shasta snow-wreath populations has grown steadily from three in 1992, to 17 in 2004, to 23 in 2012 (Lindstrand and Nelson 2005, 2006; DeWoody et al. 2012), and to 33 as of this writing (CDFW 2016; L. Lindstrand III, North State Resources, Inc. personal communication; Fig. 1). The California Natural Diversity Database (CNDDB) lists 24 "occurrences" of Shasta snow-

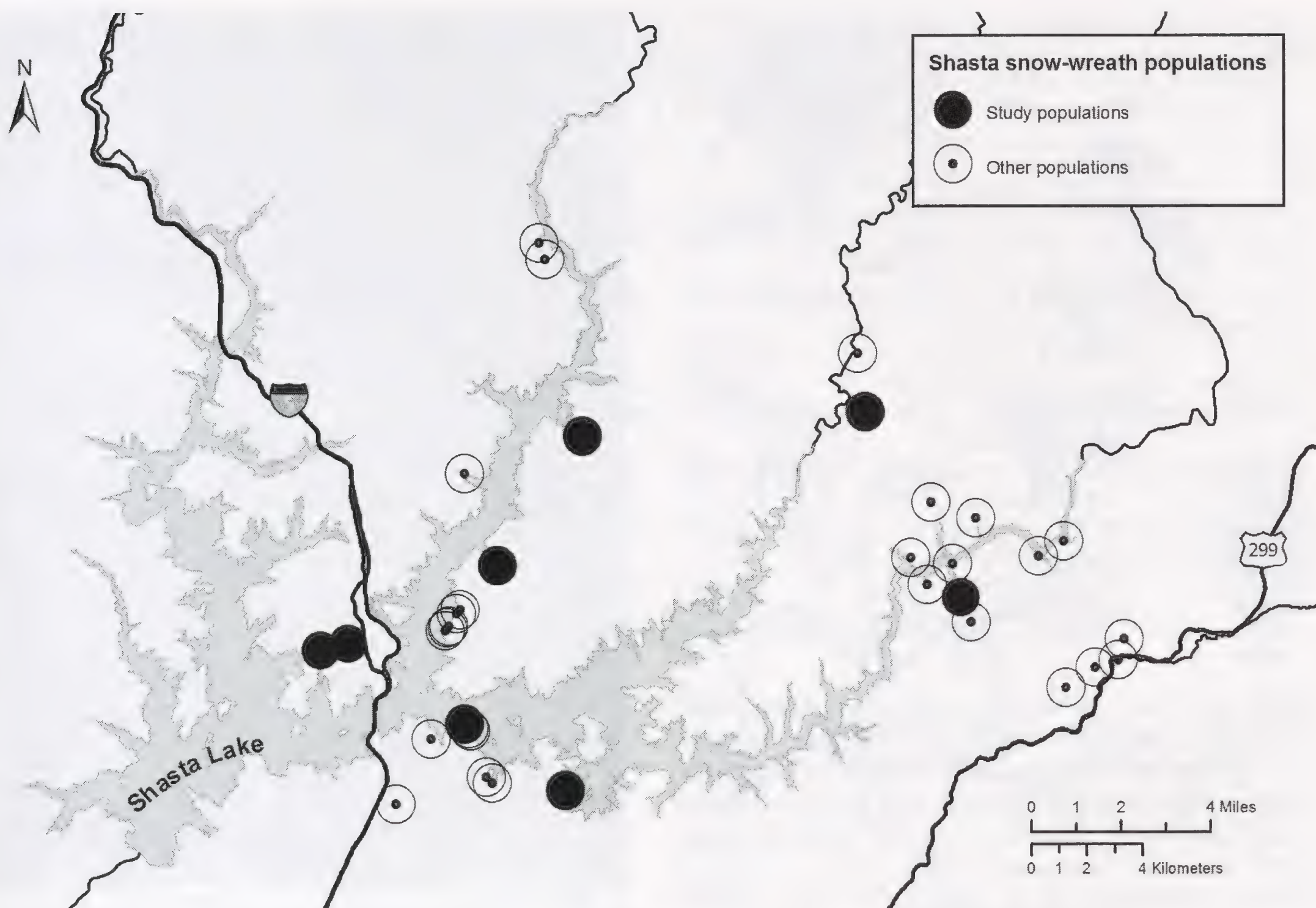


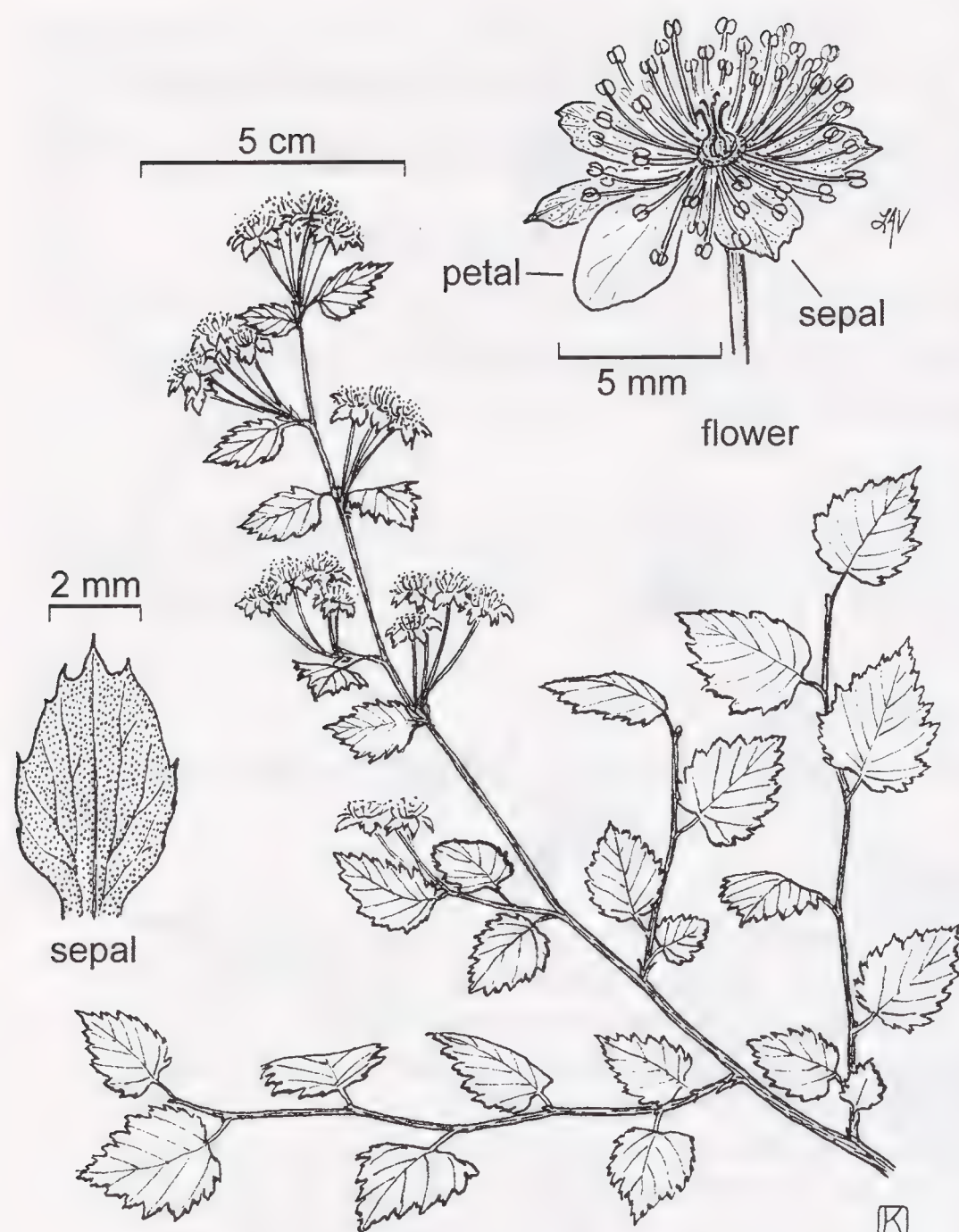
FIG. 1. Locations of all 33 known Shasta snow-wreath populations.

wreath, however an occurrence can include multiple, discrete patches found within 0.25 miles of one another if they are not separated by significant habitat discontinuity (CDFW 2016). In this paper, we term the discrete patches “populations” and use them as our focus of discussion. All of the known populations are found within the Cow Creek, McCloud River, Pit River, Sacramento River, and Squaw Creek watersheds near Shasta Lake, which was formed in 1945 when Shasta Dam was completed. Whether the formation of the lake destroyed some populations of Shasta snow-wreath and further restricted its range is unknown, but seems likely, given that populations are found directly adjacent to the lake. A plan has been proposed by the U.S. Bureau of Reclamation that would raise the height of Shasta Dam up to 5.6 m and flood additional terrestrial habitat (U.S.D.I. 2015). Previous analyses suggest this lake expansion project would submerge portions of nearly 40% the known Shasta snow-wreath populations, though only ~3% of the total habitat occupied by the species would be submerged (U.S.D.I. 2014). Nonetheless, two small populations would experience significant losses of 92% and 95% due to rising lake levels.

The life history and ecology of Shasta snow-wreath is not well studied. To date, little work has been conducted on the basic demography of Shasta snow-wreath or its response to common disturbances

such as fire and herbivory. We are aware of one study by Sierra Pacific Industries in which the response of a portion of a single Shasta snow-wreath population to timber harvesting is being monitored, though these results are not yet published (T. Engstrom, Sierra Pacific Industries personal communication).

Shasta snow-wreath is deciduous and bears flowers with showy stamens and 0–2 petals at the base (Fig. 2). Fruits are rarely observed and the species is thought to reproduce primarily vegetatively, forming thickets of stems arising from the root system (Stebbins 1993). One study (DeWoody et al. 2012) used isozyme analysis to assess genetic diversity within and among 23 Shasta snow-wreath populations and found that within-population variation was low, while among-population variation was high. Five of the populations sampled were composed of single genets and the average number of genets per population was 3.14. This supports the hypothesis that sexual reproduction is less common in Shasta snow-wreath than clonal propagation, and that gene flow among populations is quite low (DeWoody et al. 2012). Whether this evident isolation of populations is a result of recent changes due to the construction of Shasta Dam, increased geographic restriction over long periods of time, or due to another unidentified source of ecological or evolutionary pressure, is unknown. Regardless of the cause, the low number of populations, small popu-



Neviusia cliftonii

FIG. 2. Shasta snow-wreath (*Neviusia cliftonii* Shevock, Ertter & D.W. Taylor) illustration showing vegetative and reproductive structures. This rare shrub grows to a height of 2.5 m and is known from 33 populations near Shasta Lake, CA. Reprinted with permission from Baldwin et al. (2012).

lation sizes, and low within-population diversity all suggest that careful monitoring of the species is warranted (Ellstrand III and Elam 1993; Schemske et al. 1994).

The primary goal of our work was to describe population characteristics of Shasta snow-wreath, including size, density, associated vegetation, and abiotic site characteristics. Additionally, we used our data to explore the relationships between population metrics (e.g., number of stems, or ramets, per population) with site characteristics to better understand what factors might influence Shasta snow-wreath. To do this, we established permanent plots within eight of the 33 known populations. While the number of plots is relatively small, the study included detailed observations at each site. Additionally, the system of plots was created to allow for long-term monitoring efforts and for an expansion of the methodology to other populations. In particular, these plots will be used to assess population trends over time with special attention to understanding the impact of fire. In the study area, fire is particularly important as increases in temperature and climate water deficits have increased the likelihood for greater extent and frequency of fires in the region (Miller et al. 2009).

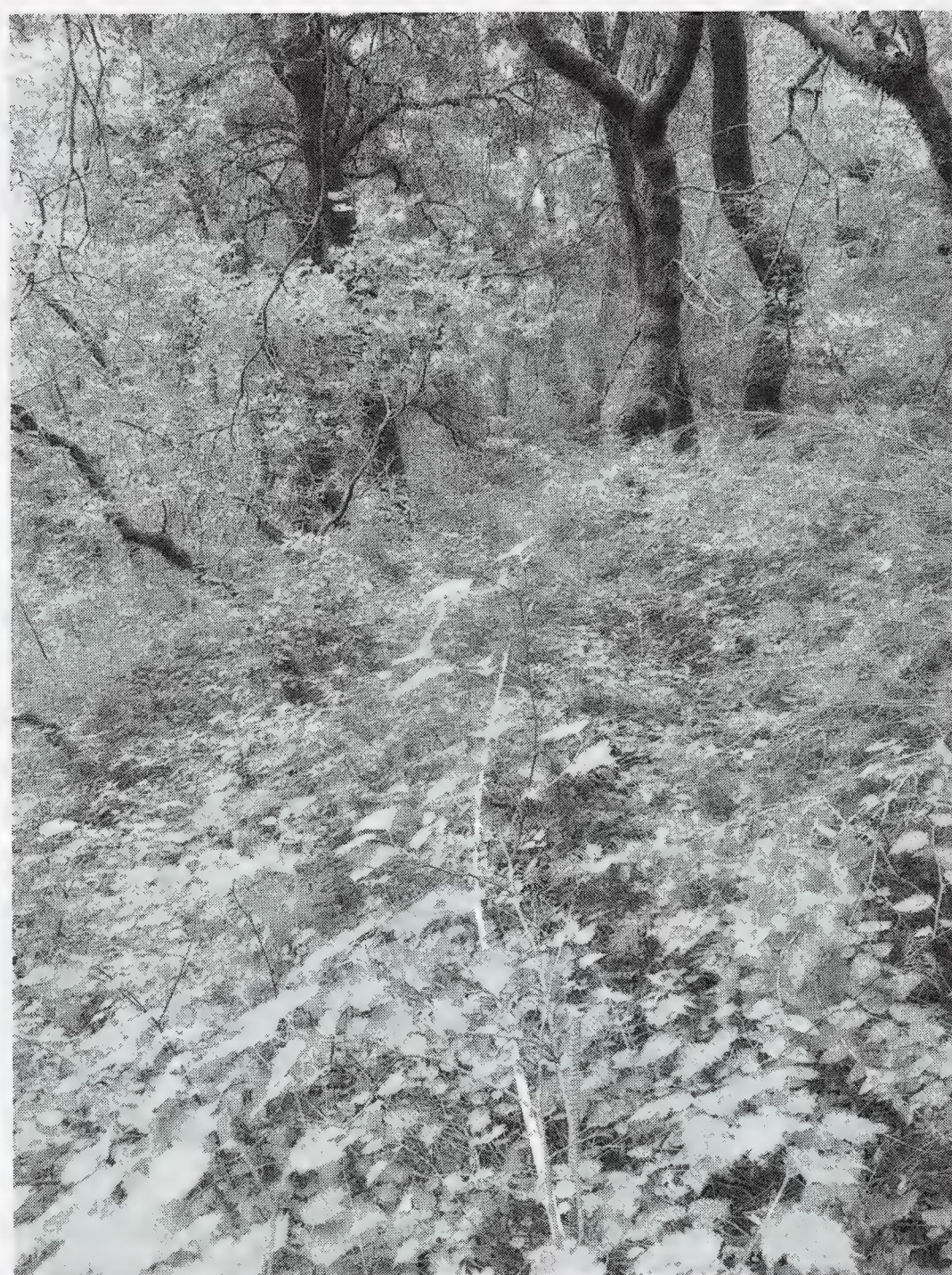


FIG. 3. Typical habitat of Shasta snow-wreath (*Neviusia cliftonii* Shevock, Ertter & D.W. Taylor) with a permanently-marked transect at the Waters Gulch site (Table 1). The dominant canopy trees shown here are canyon live oak (*Quercus chrysolepis* Liebm.). Photo courtesy H. Kurkjian.

METHODS

Data Collection

During the summers of 2011–2013, we established eight permanent transects for population surveys and future monitoring of Shasta snow-wreath (Table 1; Fig. 3). Specific sites were chosen by finding relatively large patches of Shasta snow-wreath (at least $\sim 40 \text{ m}^2$) through which we could establish a 2-m wide belt transect. Sites were selected such that they were distributed among the known populations and easily accessed along roads or by boat. Several populations were excluded because they were located on private lands or were very small (J. Nelson, U.S. Forest Service, L. Lindstrand III, North State Resources, Inc., personal communication). We positioned transects through the center of each population, recorded their length and bearing, and installed metal stakes (rebar) at both ends. We recorded plot elevation and UTM coordinates using a Trimble Nomad or Juno 3B GPS unit (Trimble, Sunnyvale, CA, USA). We also recorded topographical characteristics of each site, including the shape, position, aspect, and degree of slope.

TABLE 1. Topographical characteristics recorded at eight Shasta snow-wreath populations. Population names (“Site”) correspond to those found in DeWoody et al. 2012 (with the exception that our study used Waters Gulch twice). “CNDDDB element #” lists the California Natural Diversity Database (CNDDDB) element occurrence number. “Order 3 soil type” is from USDA Forest Service (1983) and “Geologic soil type” is from USDA Forest Service (2004).

Site	CNDDDB element #	Elev. (m)	Aspect	Slope (°)	Slope position	Order 3 soil type	Geologic soil type
Blue Ridge	16	332	22	35	Backslope	Holland	Shale; siltstone; metavolcanic; w/ limestone; & undiff.
Campbell Creek	6	336	22	21	Toeslope	Holland/Goulding	Tuffaceous mudstone w/lesser mafic flows; sandstone; cherty quartzite; other ms; & undiff.
Curl Creek	5	397	16	35	Backslope	Holland	Greenstone & greenstone breccia
Jones Valley	21	323	20	35	Footslope	Marpa Holland	Shale; siltstone; metavolcanic; w/ limestone; & undiff.
Low Pass	7	473	240	10	Shoulder	Marpa-Neuns	Limestone; thin-bedded to massive; gray; fossiliferous
Stein Creek	12	332	55	65	Backslope	Neuns	Andesitic volcanoclastic & pyroclastic rocks; conglomerate; & undiff.
Waters Gulch	14	311	18	28	Backslope	Holland	Greenstone; & undiff.
Waters Gulch 2	14	425	323	65	Shoulder	Holland	Greenstone; & undiff.

Of the eight total plots, we established seven in 2011 and added one additional site (Waters Gulch 2) in 2012 (Table 1). Although we had anticipated sampling all eight populations in 2012 and 2013, low water levels in Shasta Lake made accessing some of the sites difficult because they are most easily reached by boat. Thus, we were unable to sample Campbell Creek in 2012 and Stein Creek in 2013. The U.S. Forest Service performed a small prescribed burn in December 2011 which partially burned the Jones Valley population, including 50% of our transect. We were unable to assess the precise response of Shasta snow-wreath to the fire because soil slumping near the base of the slope shifted the rebar used to mark the exact start location of our transect at that site. However, we did flag burned patches immediately post-fire and we report on our qualitative observations of fire effects from the site in 2012 and 2013.

In each population we characterized Shasta snow-wreath by sampling in 1 m quadrats along both sides of the entire transect, forming a belt transect that was 2 m wide. In each quadrat, we recorded the number of Shasta snow-wreath stems, the number of inflorescences per stem, and the height of the tallest stem. We also recorded the width of the population at the initial transect origin, at each 5 m increment along the transect, and at the transect endpoint. These measures were used to determine the total area of the population. We also characterized other species associated with Shasta snow-wreath populations by recording the rank order of dominant trees and, separately, shrubs found within the population area. We also sampled each belt transect for ground and vegetative cover. Ground cover measurements included the percent cover bare ground (particles < 2 mm), gravel (particles 2–15 mm), rock (particles > 15 mm), woody debris (stems > 7.62 cm diameter), litter (stems < 7.62 cm diameter), and total basal vegetation (all live plants combined). Vegetative cover measurements included the percent cover of

live and dead trees, live and dead shrubs, forbs, ferns, moss, grass, and lichen. We also recorded the percent cover of all invasive plant species (defined by Cal-IPC 2006) in the belt transect and noted any invasive species present in the remainder of the population. Lastly, we extended a separate Brown’s Fuels transect (Brown 1974) in a randomly selected direction from the belt transect origin at each site and recorded the number of 1-, 10-, 100-, and 1000-hr fuels. At each 5 m length of the fuels transects we recorded litter and duff depth.

Analyses

For each site, we averaged all observations across all years that were sampled. We did this for two reasons: (1) we did not have a record of all sites in all years and, (2) site- and population-level data did not vary considerably from year to year. We then summarized each plot’s topography, vegetation, and Shasta show-wreath population characteristics. We calculated the mean Shasta snow-wreath stem density, number of inflorescences per stem, and average maximum height. We then estimated the total number of stems in each population by scaling the density of stems within the belt transect to the total population area. Because multiple stems may belong to a single genet, we consider our stem counts to be ramet counts. That is, rather than estimating “population sizes” per se, we estimated “ramet population sizes” and use that terminology throughout. Individual sites were averaged across years for vegetation and ground cover metrics as well as fuel loads (see Tables 2 and 3). We transformed aspect to linear aspect using the equation

$$lASP(i) = 1 + \cos \frac{\pi(aspect(i) - 45)}{180}.$$

where $lASP(i)$, and $aspect_i$, are the linear aspect and aspect of the site i , respectively. The transformation

TABLE 2. Summary statistics for eight Shasta snow-wreath populations. All values are averages across all years for which observations were made in belt transects, except for two categories: “Population area” is the estimated total geographic area of the population, and “Ramet population size” is the number of stems in the estimated population area. See Methods section for details.

Site	Years surveyed	Transect length (m)	Population area (m ²)	Number of inflorescences	Average number of inflorescences per stem	Average maximum height (m)	Number of stems counted	Ramet population size
Blue Ridge East	3	20	365.5	569	0.98	115	566	4585
Campbell Creek	2	25	232.9	733	3.34	92	209	1022
Curl Creek	3	29	310.3	329	1.02	69	317	1729
Jones Valley	3	20	410.8	30	0.08	50	359	3878
Low Pass	3	25	1366.7	3391	7.92	142	417	11,708
Stein Creek	2	20	129.4	1089	4.91	73	215	716
Waters Gulch	3	9	86.3	230	0.75	129	292	1459
Waters Gulch 2	2	25	1007.6	2830	3.06	159	847	18,641

results in values that range from 0.0 (warmer, drier southwest-facing slope) to 2.0 (cooler, moister northeast-facing slopes). To determine if any of the demographic characteristics of Shasta snow-wreath populations are associated with site characteristics, we used Spearman’s rank correlations. Specifically, we assessed correlations between topographical, vegetation and ground cover characteristics and the estimated Shasta snow-wreath ramet population size, average maximum height (cm), and number of inflorescences per stem. In addition, we described the dominant vegetation associated with Shasta snow-wreath by summing the ranking of dominant trees and shrubs associated with each site across all years.

RESULTS

The eight Shasta snow-wreath populations we studied occur from 332 m to 473 m elevation and on slopes of varying steepness (10° to 65°, Table 1). Most populations occur on north-facing slopes and on the backslope positions (Table 1). The permanently marked transects established in each population ranged from 9 m to 29 m (mean = 21.6 m). The number of stems observed in these transects varied from 160 to 932, and, when averaged across the years sampled for each population, varied from 220 to 925 (Table 2). The geographical area of populations

varied from 86 m² to 1367 m² (mean = 489 m², Table 2). The average Shasta snow-wreath population we sampled consisted of an estimated 5467 stems (range = 716 to 18,641, Table 2). Populations also differed with respect to the number of inflorescences; the number of inflorescences ranged from 0 to 4231, and averages across years for each site ranged from 30 to 3391 (Table 2). The average maximum height of Shasta snow-wreath was 104 cm and ranged from 50 to 159 cm (Table 2).

All sites were characterized by relatively high cover of live shrubs (51% to 96%) and live trees (68% to 95%). The percent cover of other vegetation (forbs, ferns, grass, and lichen) was relatively low among all sites (<10%; Table 3), although, the cover of moss varied between sites with the greatest cover observed at Campbell Creek (31%) and Stein Creek (87.5%; Table 3). The study sites included very low cover of dead shrubs (0.25% to 1.25%) and dead trees (0.25% to 1.33%; Table 3). At most sites the ground was mostly comprised of litter (41.5% to 97.66%) and in some cases moss (see Campbell Creek and Stein Creek). We observed low cover of bare ground (0.25% to 2.83%), rock (0.16% to 1.75%), woody debris (1% to 7.33%) and basal vegetation (1% to 2.33%). We observed the invasive plant Himalayan blackberry (*Rubus armeniacus* Focke) in three of the eight sites (Blue Ridge, Low Pass, and Campbell Creek) where its cover reached as

TABLE 3. Average percent vegetative cover recorded at each site across all survey years (2011–2013). Vegetation cover metrics included the percent ground cover and percent vegetation cover on other species (e.g., the percent cover moss category includes cover on the ground and other organisms).

Site	Live Tree	Dead Tree	Live shrub	Dead shrub	Forb	Fern	Moss	Grass	Lichen	Bare ground	Rock	Woody Debris	Basal Veg.	Litter
Blue Ridge East	75	0.66	97	1.25	0.75	0.5	7.75	0.5	0.25	1	0.5	2	1.5	92.66
Campbell Creek	95	0.5	94	0.25	1.25	1.25	31	2.25	0.5	0.5	0.75	3.5	2	59
Curl Creek	88	0.33	91.33	1	1.5	2.16	3.83	0.16	0.33	0.5	0.5	2.66	1.5	94.16
Jones Valley	88.3	1.33	51.66	0.5	0.66	1	1	0.16	0.16	2.83	0.5	1	1.33	95
Low Pass	68.3	1.33	96.33	0.5	2.16	0.16	2.66	0.5	0.5	0.5	0.16	7.33	2.33	88
Stein Creek	90	0.25	85	4	5.25	7.5	87.5	0.5	0.5	1	0.5	5	2	41.5
Water’s Gulch	86.7	0.5	88	0.5	1.66	0.33	0.5	0.66	0.16	0.16	0.33	1.33	1	97.66
Water’s Gulch 2	77.5	0.25	94.5	1	0.5	0.25	1	0.25	0.5	0.25	1.75	1.25	1.25	95.5

TABLE 4. Ranking of the 15 dominant tree and shrub species associated with eight Shasta snow-wreath populations across all sites and all years. Species were ranked at each site (e.g., 1, 2, 3, etc. . .) and those species with the lowest sum across sites are ranked as most abundant. See text for details.

Rank	Dominant Trees	Dominant Shrubs
1	<i>Quercus kelloggii</i> Newb.	<i>Neviusia cliftonii</i> Shevock, Ertter & D.W. Taylor
2	<i>Pseudotsuga menziesii</i> (Mirb.) Franco	<i>Toxicodendron diversilobum</i> (Torr. & A. Gray) Greene
3	<i>Acer macrophyllum</i> Pursh	<i>Rosa</i> spp.
4	<i>Quercus chrysolepis</i> Liebm.	<i>Styrax redivivus</i> (Torr.) L.C. Wheeler
5	<i>Pinus ponderosa</i> P. Lawson & C. Lawson	<i>Aralia californica</i> S. Watson
6	<i>Quercus wislizeni</i> A. DC.	<i>Philadelphus lewisii</i> Pursh
7	<i>Umbellularia californica</i> (Hook. & Arn.) Nutt.	<i>Symphoricarpos albus</i> (L.) S.F. Blake
8	<i>Quercus garryana</i> Douglas ex. Hook.	<i>Rubus</i> spp.
9	<i>Cornus</i> spp.	<i>Cornus</i> spp.
10	<i>Aesculus californica</i> (Spach) Nutt.	<i>Staphylea bolanderi</i> A. Gray
11	<i>Pinus sabiniana</i> Douglas ex. G. Don	<i>Berberis</i> spp.
12	<i>Alnus rhombifolia</i> Nutt.	<i>Ribes</i> spp.
13	<i>Fraxinus latifolia</i> Benth.	<i>Corylus cornuta</i> Marshall
14	<i>Pinus attenuata</i> Lemmon	<i>Frangula californica</i> (Eschsch.) A. Gray
15	<i>Salix</i> spp.	<i>Calycanthus occidentalis</i> Hook. & Arn.

high as 7.5% (Low Pass). Six of our eight populations were found on north-northeast facing to northwest-facing aspects (between 16° and 55°), while Low Pass and Waters Gulch 2 were found on northwest-facing and southwest-facing aspects, respectively (Table 5). We found that the eight Shasta snow-wreath study populations had, on average, 81,337 kg ha⁻¹ dry biomass and most of this fuel (~60%) was comprised of fine fuels (<7.6 cm diameter; 1-, 10-, 100-hr fuels). Fine fuels, which are associated with wildfire risk, ranged from 5422 kg ha⁻¹ to 134,064 kg ha⁻¹ among our populations (mean = 48,624 kg ha⁻¹).

We recorded 17 tree and 36 shrub species that were associated with Shasta snow-wreath (Table 4). The most common tree species we recorded were California black oak (*Quercus kelloggii* Newb.), Douglas-fir (*Pseudotsuga menziesii* Mirb.), big-leaf maple (*Acer macrophyllum* Pursh), and canyon live oak

(*Quercus chrysolepis* Liebm.), while the most common shrubs were the Shasta snow-wreath, poison oak (*Toxicodendron diversilobum* [Torr. & A. Gray] Greene), roses (*Rosa* spp.), and snowdrop bush (*Styrax redivivus* [Torr.] L.C. Wheeler; Table 4).

A low-intensity prescribed burn at the Jones Valley site in December 2011 burned approximately 50% of the transect. We observed basal resprouting (Fig. 4) and evidence of deer browse throughout the burned portion of the population during resampling in summer 2012. In small patches where the shrubs appear to have been top-killed by the burn, resprouting was still evident. Herbivory was limited to the new growth.

The eight Shasta snow-wreath populations that we studied varied considerably in both vegetative and reproductive characteristics. Some of this variation, but not all, could be explained by site characteristics we measured. We found a significant correlation between aspect and the estimated number of inflorescences per stem ($r_s = 0.76$, $p = 0.028$, Table 5); the least number of inflorescences per stem was found in the four most north-facing sites (16–22°, Table 2). Additionally, we found a marginally significant increase in the number of inflorescences at higher elevations ($r_s = 0.66$, $p = 0.075$) and a significant increase in sites with a higher percent cover of lichen ($r_s = 0.82$, $p < 0.014$; Table 5). We have no measure of precipitation at our study sites, although precipitation may increase in elevation and be a more proximal cause of more inflorescences. Average maximum height (cm) of Shasta snow-wreath was greatest in sites with the smallest percent cover of live trees, though this relationship was marginally significant ($r_s = -0.69$, $p = 0.058$). Also, the relationship of average maximum height and aspect was marginally significant ($r_s = -0.63$, $p = 0.094$); the same sites that had high inflorescence density had tall stems (Table 2). The estimated ramet population sizes were significantly larger in sites at higher elevations ($r_s = 0.74$, $p = 0.036$) and with lower

TABLE 5. Spearman’s non-parametric correlations between site cover characteristics and individual and population-level Shasta snow-wreath characteristics including the average number of inflorescences per stem, average maximum height (m), and estimated ramet population size. ** denotes significant associations ($\alpha = 0.05$) and * denotes marginally significant associations ($0.05 < \alpha < 0.1$).

	Inflorescences per stem		Average maximum height (m)		Ramet population size	
	r_s	P	r_s	P	r_s	P
Slope	-0.21	0.772	-0.04	0.918	0.21	0.611
Aspect	-0.76	0.028**	-0.63	0.094*	-0.71	0.045**
Elevation	0.66	0.075*	0.52	0.183	0.74	0.036**
Fern	-0.2	0.615	-0.51	0.196	-0.44	0.278
Forb	0.5	0.212	-0.25	0.893	-0.54	0.389
Grass	0.17	0.683	-0.01	0.996	-0.32	0.435
Live Tree	-0.42	0.292	-0.69	0.058*	-0.72	0.050**
Dead Tree	0.16	0.7	-0.09	0.862	0.11	0.791
Lichen	0.82	0.014**	0.3	0.468	0.39	0.336



FIG. 4. Evidence of resprouting on Shasta snow-wreath (*Neviusia cliftonii* Shevock, Ertter & D.W. Taylor), seven months after a 2011 prescribed burn at the Jones Valley site (Table 1). The dominant canopy trees shown here are canyon live oak (*Quercus chrysolepis* Liebm.). Photo courtesy R. Butz.

cover of live trees ($r_s = -0.71$, $p = 0.05$). Likewise, the most north-facing sites had the smallest estimated number of ramets ($r_s = -0.72$, $p = 0.045$; Table 5). We note that when evaluating these correlations, we have not adjusted the level of significance (α) to reduce the risk of Type I errors when using multiple comparisons. For instance, a Bonferroni adjustment would result in $\alpha = 0.0019$ ($0.05 \div 27$ tests). Instead, we present unadjusted P -values as a means of exploring potential hypotheses about Shasta snow wreath distribution and limit our conclusions based on the relatively small size of the study.

DISCUSSION

Overall, the population structural information we collected suggests some interesting hypotheses about the factors that control Shasta snow-wreath populations. Our largest ramet population sizes, and our tallest individuals, were found in sites with relatively low canopy cover of live trees (Tables 2 and 5). This relationship is intriguing and suggests that removal of the canopy by fire may have been a critical disturbance for this rare shrub. The California black oak woodlands and Pacific ponderosa pine – Douglas-fir forests (Eyre 1980) where Shasta snow-

wreath populations occur exhibit very high departures from pre Euro-American settlement fire frequencies (Safford and Van de Water 2014) and the presence of relatively fire-intolerant Douglas-fir in the overstory is indicative of prolonged fire suppression. Historically, this vegetation experienced frequent wildfires with an average fire return interval of 12 years (Taylor and Skinner 2003; Fry and Stephens 2006; Safford and Van de Water 2014). Restoring a more frequent fire return interval through prescribed burning or employing a mechanical fuels treatment to reduce canopy cover may benefit Shasta snow-wreath. However, we do not know how Shasta snow-wreath would respond to higher severity wildfire under these fire-suppressed conditions where fuel loads may be elevated. Our measures of current fuel loads should be useful for long-term monitoring.

Lastly, the two largest populations (Low Pass and Waters Gulch 2) were notable in that they were at relatively high elevations and their aspects were westerly-facing, while the other six populations were all found in a small range of aspects (16° to 55° ; Table 1). These same populations tended to have more inflorescences per stem as compared with other populations. However, the relationship of aspect and population characteristics is confounded by a positive relationship between live canopy and aspect ($r_s = 0.78$, $p = 0.022$; Table 5). Thus, expansion of our monitoring program to include more of the other 33 populations—across a broad range of aspects—would help elucidate the roles of canopy, aspect, and other site characteristics. Also, several additions should be made to the monitoring program. Himalayan blackberry occurred in three of our eight sites and was common in the general study area, so assessing its impact on Shasta snow-wreath across a larger number of populations is warranted. Finally, little is known about the potential impacts of herbivores, pathogens and pollinators on Shasta snow-wreath and these are worth pursuing in future work. Overall, this monitoring effort has provided a baseline from which to pursue repeat measure of existing permanent plots and a basis for expansion of studying other Shasta snow-wreath population characteristics.

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NUCLEAR RIBOSOMAL DNA REVEALS INFRASPECIFIC VARIATION IN *CORETHROGYNE FILAGINIFOLIA* (ASTERACEAE)

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ABSTRACT

Corethrogyne DC. (Asteraceae) is a controversial genus due to complex and confounding variability, and the subject of multiple taxonomic revisions. It was previously included within *Lessingia* Cham. The single species, *C. filaginifolia* Nutt., has high infraspecific variability. This study was conducted to better understand this infraspecific variability for *C. filaginifolia* var. *incana* (Lindl.) Canby and *C. filaginifolia* var. *linifolia* H. M. Hall. Barcoding loci from nuclear ribosomal DNA (nrDNA) and chloroplast DNA (cpDNA) were used along with morphological comparisons of voucher specimens. Based on two nrDNA loci, maximum parsimony, maximum likelihood, and Bayesian analyses, we detected five haplotypes of *C. filaginifolia*. The two focal varieties of this study, *C. filaginifolia* var. *incana* and *C. filaginifolia* var. *linifolia*, were indistinguishable at both, nrDNA and cpDNA loci.

Key Words: Asteraceae, California, DNA barcoding, *Lessingia*.

Asteraceae is one of the largest families of Angiosperms with more than 23,000 species (Panero and Funk 2008). Rapid diversification approximately 30 million years ago resulted in high morphological, chemical, and biological diversity (Jensen and Palmer 1987). Phylogenetic relationships at the tribal and subfamily levels within the Asteraceae have been examined extensively (Funk et al. 2009), though confusion still persists for some major lineages (Panero and Funk 2008). *Corethrogyne* DC., commonly known as sandaster, is one of several controversial genera in the Asteraceae. Its distribution is restricted to parts of the western United States (California and southwestern Oregon) and Mexico (northern Baja California) (Markos and Strother 2006; Baldwin et al. 2012) (Fig. 1a).

The taxonomy of *Corethrogyne* has been revised frequently. In 1959, Keck first proposed three species in the genus. By 1960, however, Ferris suggested seven species. Munz followed in 1974 to clarify the taxonomy of *C. filaginifolia* Nutt. using only plant material from southern California, and presented 11 varieties. Approximately two decades later, Lane (1992, 1993) unified the genus *Corethrogyne* with *Lessingia* Cham. based on morphological and chloroplast DNA similarities. Between 1993 and late 1990s, various studies (Zhang and Bremer 1993; Nesom 1994; Morgan 1997) showed that *Lessingia* is closely related to *Corethrogyne* based on morphology and chloroplast DNA. More recently, however, Saroyan et al. (2000) published a revision presenting *Corethrogyne* as distinct from *Lessingia* based on the perennial life cycle and radiate inflorescence heads in *Corethrogyne* as opposed to an annual life cycle and sometimes discoid inflorescence in *Lessingia*. Notably, the same authors also reported morphometric distinctiveness between northern and southern populations of *C. filaginifolia* in California based on the

number of inflorescences per flowering stem and length:diameter ratio of the involucre. Subsequently, in 2001, Markos and Baldwin provided genetic evidence to support the discrimination between *Lessingia* and *Corethrogyne*. In 2012, Markos and Strother recognized a single species of *Corethrogyne*, *C. filaginifolia* Nutt., without any infraspecific taxa. Simultaneously, California Native Plant Society (CNPS 2015) maintains two species within the genus *Corethrogyne* (*C. leucophylla* Jeps. and *C. filaginifolia*) and recognizes two varieties of *C. filaginifolia*: 1) *C. filaginifolia* Nutt. var. *incana* (Lindl.) Canby, and 2) *C. filaginifolia* Nutt. var. *linifolia* H.M. Hall. Adding to the confusion further, CalFlora (2015) also recognizes the same two species (*C. leucophylla* and *C. filaginifolia*), but maintains four varieties within *C. filaginifolia*: 1) *C. filaginifolia* Nutt. var. *californica* (DC.) Saroyan, 2) *C. filaginifolia* Nutt. var. *filaginifolia*, 3) *C. filaginifolia* var. *incana*, and 4) *C. filaginifolia* var. *linifolia*. The three *Corethrogyne* taxa recognized by CNPS are also listed in the rare, and endangered plant inventory of CNPS (2015), but considering the taxonomic uncertainty within the genus and within the *C. filaginifolia* complex in particular, the conservation status of these taxa remains unclear (Markos and Baldwin 2001).

Two of the recognized taxa, *C. filaginifolia* var. *incana* and *C. filaginifolia* var. *linifolia*, are given a rarity rank of 1.B1 (CNPS 2015). Both taxa are endemic to San Diego County and occur in coastal scrub and chaparral, but occupy different areas within this narrow geographic range (Munz 1974; CNPS 2015) (Fig. 1b). *Corethrogyne filaginifolia* var. *incana* is restricted to sandy soils of the Point Loma peninsula. *Corethrogyne filaginifolia* var. *linifolia* occurs on open bluffs and in shrubby habitats of Del Mar.

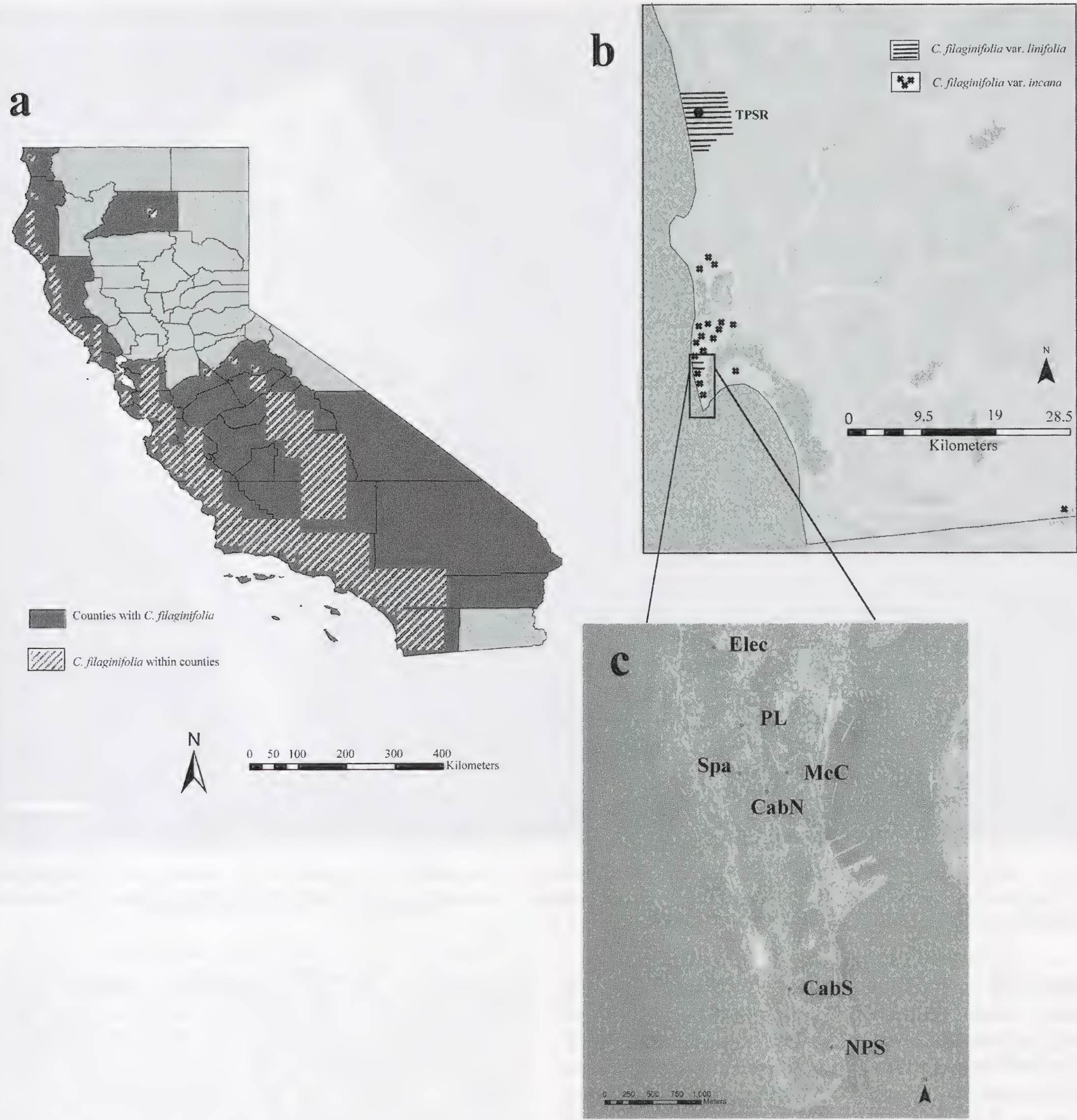


FIG. 1. Maps showing the distribution of *Corethrogyne filaginifolia* and locations where individuals were sampled for this study. a) A map of California showing the counties hosting *C. filaginifolia* populations, and coarse distribution of *C. filaginifolia* within them indicated by hatching (CalFlora 2015). b) A map of San Diego County showing the approximate distribution of *C. filaginifolia* var. *incana* and *C. filaginifolia* var. *linifolia* (also from CalFlora 2015). Additionally, a sampling location within Torrey Pine State Reserve (TPSR) is shown where *C. filaginifolia* var. *linifolia* was sampled. c) A map of Point Loma peninsula showing locations where the two varieties of *C. filaginifolia* were sampled; Cabrillo Drive (CabN, CabS), McClelland Road (McC), SPAWAR (Spa), Electron Drive (Elec), and National Park Service (NPS) represent the sampling sites for *C. filaginifolia* var. *incana*, while the location coded as Point Loma (PL) represent the sampling site for *C. filaginifolia* var. *linifolia*.

However, both taxa have been reported (as collections and occurrences) from outside these core ranges (CalFlora 2015). CNPS (2015) reported that the distribution of *C. filaginifolia* var. *linifolia* extended to Rancho Santa Fe, La Jolla, Del Mar, Encinitas and San Luis Rey, and the distribution of *C. filaginifolia* var. *incana* included National City, Point Loma, Imperial Beach, Del Mar, La Jolla, and Rancho Santa Fe (Fig. 1b). Because both taxa (*C. filaginifolia* var.

incana and *C. filaginifolia* var. *linifolia*) are present on the Point Loma peninsula where they may potentially hybridize, conservation concerns arose with respect to their taxonomic uniqueness.

Corethrogyne filaginifolia var. *incana* can be distinguished morphologically from *C. filaginifolia* var. *linifolia* by presence of distinct stout-stalked glands (Fig. 2a) on the involucre bracts and distal plant parts making them sticky to touch. In comparison, *C.*

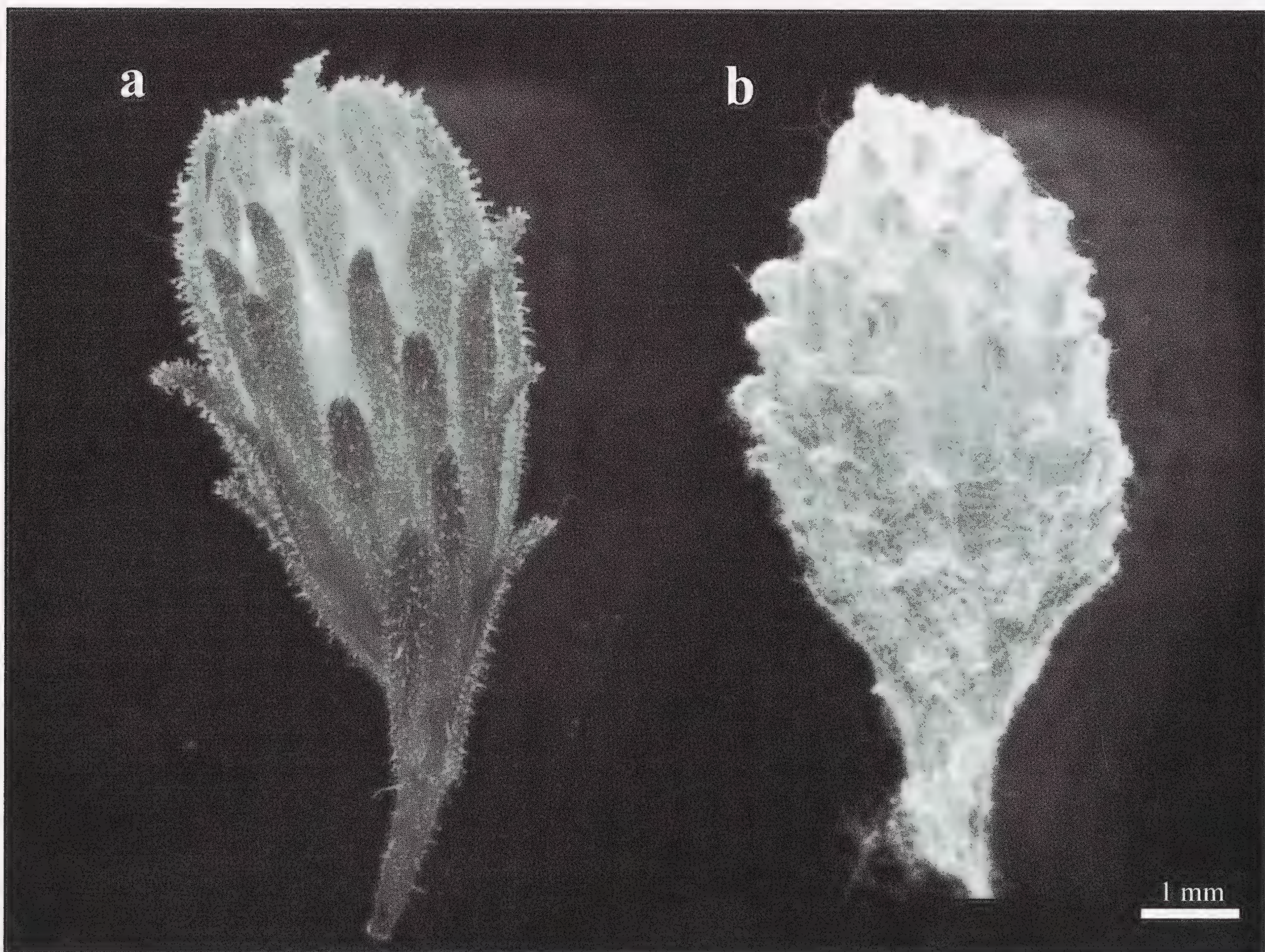


FIG. 2. Morphological differences between involucral bracts of *Corethrogyne filaginifolia* var. *incana* and *C. filaginifolia* var. *linifolia* are shown. a) Involucral bracts of *C. filaginifolia* var. *incana* are covered with stout-stalked glands. b) Involucral bracts of *C. filaginifolia* var. *linifolia* have tomentose surfaces.

filaginifolia var. *linifolia* has tomentose involucral bracts, lacking glands (Munz 1974) (Fig. 2b). Munz (1974) reported additional morphological and phenological differences to discriminate between the two taxa which included; 1) plant height (*C. filaginifolia* var. *incana* = 50–80 cm, *C. filaginifolia* var. *linifolia* = 20–40 cm), 2) involucre length (*C. filaginifolia* var. *incana* = 10–12 mm, *C. filaginifolia* var. *linifolia* = 8–10 mm), and 3) leaf shape (*C. filaginifolia* var. *incana* = linear to narrowly lanceolate, *C. filaginifolia* var. *linifolia* = narrow with 1–2 mm width). Further, genetic differences between the two varieties have not been investigated to test the taxonomic distinctiveness.

We sought to assess whether the two varieties, *Corethrogyne filaginifolia* var. *incana* and *C. filaginifolia* var. *linifolia*, could be distinguished using multiple DNA barcoding loci (Hebert et al. 2003). Toward this end, we compared the two taxa using six genomic loci that have previously been used to discriminate taxa within the Asteraceae and in other plant families (Chase et al. 2007; Kress et al. 2007; CBOL Plant Working Group 2009; Chen et al. 2010; Gao et al. 2010).

Further, to broadly compare with the Californian *C. filaginifolia* complex, we combined our data

generated in this study (without vouchered specimens) from the two varieties with sequence data (with vouchered specimens) previously generated by Markos and Baldwin (2001, 2002) and from NCBI (PopSet 417346869). We hypothesized that: 1) *Corethrogyne filaginifolia* var. *incana* and *C. filaginifolia* var. *linifolia* are genetically distinguishable from each other at the selected barcode loci. We based this hypothesis on the observations of Munz (1974) and our personal observation of the morphological differences between the two varieties; and 2) individuals within the Californian *C. filaginifolia* complex show sub-specific variation based on a latitudinal gradient. This hypothesis was based on the morphometric study of Saroyan et al. (2000), which suggested the distinction between northern and southern populations of *Corethrogyne* in California.

MATERIALS AND METHODS

Sample collection and DNA extraction

Three individuals of *C. filaginifolia* var. *incana* were sampled from each of the following six

locations: Cabrillo Drive (CabN, CabS), McClelland Road (McC), SPAWAR (Spa), Electron Drive (Elec), and National Park Service (NPS) on Point Loma peninsula, San Diego County (Fig. 1c). The sampled sites for *C. filaginifolia* var. *incana* were within approximately 0.5–5 km from each other.

Three individuals belonging to *Corethrogyne filaginifolia* var. *linifolia* were sampled at each of the following two locations: Point Loma (PL) (a central site on the peninsula) and Torrey Pines State Natural Reserve (TPSR) in Del Mar, San Diego County (Fig. 1b and c). TPSR is approximately 25–26 km from PL.

Up to 10 leaves were collected from each plant in May 2014 in order to obtain DNA for the barcode analyses. Plants were collected at reproductive stage when *C. filaginifolia* var. *incana* can be easily distinguished from *C. filaginifolia* var. *linifolia* by the presence of stout-stalked glands on its involucre bracts (Fig. 2). No plant materials were collected for herbarium voucher specimens. Leaf samples were stored at 4°C immediately upon collection.

Leaves from each individual were washed and sliced into 1–2 mm sections upon arrival at the laboratory in Texas Tech University, Lubbock, TX, to prepare them for DNA extraction. DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) was used to obtain DNA following the manufacturer's protocol. DNA quantity and quality was checked using NanoDrop ND1000 (Thermo Scientific, Waltham, MA, USA) and confirmed by running 1 µl genomic DNA on a 1% agarose gel.

PCR Amplification and Sequencing

For *C. filaginifolia* var. *incana* and *C. filaginifolia* var. *linifolia*, we used four cpDNA (*psbA-trnH*, *rbcL*, *rpoC1*, *ycf5*), and two nrDNA (ITS and ITS2) plant barcoding loci that have previously been used to discriminate taxa within the Asteraceae and in other plant families (Chase et al. 2007; Kress et al. 2007; CBOL Plant Working Group 2009; Chen et al. 2010; Gao et al. 2010). These loci were amplified via polymerase chain reaction (PCR) in a Mastercycler EPGradient (Eppendorf, Hamburg, Germany) using genomic DNA. Primer sequences and PCR thermal profiles were obtained from Chen et al. (2010). For each reaction, a 25 µl mixture was prepared by using the Promega GoTaq Flexi DNA Polymerase reagent kit (Promega, Madison, WI, USA). Reagent concentrations were 5 µl of 5× Green GoTaq Flexi Buffer, 0.4 µl of 40 mM dNTPs (10 mM of each dNTP), 4 µl of 25 mM MgCl₂, 1 µl of each primer (10 µM), 0.25 µl of 10 µg/µl bovine serum albumin, 0.25 µl of 5U/µl GoTaq DNA polymerase, 4 µl of 50–100 ng/µl DNA, 9.1 µl of sterile H₂O. During optimization, PCR was first performed by using the thermal profiles recommended by Chen et al. (2010). The loci that did not amplify or amplified inconsistently were exposed to touchdown PCR by adding a ±5°C range to the annealing temperature recommended by Chen et al.

(2010) for each primer. Amplicons were purified using DNA Clean and Concentrator 5 kit (Zymo Research, Irvine, CA, USA) and were sent for bi-directional sequencing in an ABI 3730xl genetic analyzer (Applied Biosystems, Foster City, CA, USA) at the DNA Analysis Facility on Science Hill, Yale University, New Haven, CT.

We then generated consensus sequences by using SeqTrace version 0.9.0 (Stucky 2012). Only reads with >100 bp length, and showing >50% overlap between forward and reverse primer reads were selected for further analyses. Quality trimming and base calling of the consensus sequences was subsequently performed using the same software. The ends of consensus sequences were trimmed if they had at least two nucleotide bases exhibiting < 20 Phred quality score (Q) using 20 bp window.

Reference Sequence Extraction from GenBank

Previously generated nrDNA sequences of 3'ETS (30 sequences), 5'ETS (5 sequences), and ITS loci (31 sequences) from voucher plants of *C. filaginifolia* (Benson et al. 1999; Markos and Baldwin 2001, 2002; NCBI PopSet 417346869) were included in the phylogenetic analyses (Table 1). We also included 3'ETS and ITS sequences from *Lessingia arachnoidea* Greene and *L. glandulifera* A. Gray to represent genera closely related to *Corethrogyne* (Markos and Baldwin 2001; Table 1). Additionally, previously generated cpDNA sequences from *Bidens pilosa* were included to represent an outgroup for *psbA-trnH*, *rbcL*, *rpoC1*, and *ycf5* (Chen et al. 2010).

Sequence Alignment and Sequence Divergence

All sequences were aligned, and consistency scores for the alignments were estimated in T-Coffee version 11.00 (Notredame et al. 2000) with M-Coffee mode. The alignments of nrDNA and cpDNA loci were concatenated separately and partitioned into data blocks where each data block represented an individual locus. The protein coding loci *rbcL*, *rpoC1*, and *ycf5* were further partitioned based on the codon position. The partitioned datasets were used to predict the best model of DNA evolution in Partition Finder version 1.1.1 using Bayesian Information Criterion (BIC) score (Lanfear et al. 2012). Sequence divergences were determined for each aligned locus using Kimura 2-parameter (K2P) DNA substitution model with gamma distribution after pairwise deletion of gaps in MEGA (Molecular Evolutionary Genetics Analysis) version 6.0 (Tamura et al. 2013).

Phylogenetic Analyses

Maximum parsimony (MP) trees were generated in PAUP* test version 4.0a146 (Swofford 2003) with a heuristic search and tree bisection-reconnection (TBR) algorithm of branch swapping. Sequence addition was random, and all characters were given

an equal weight. The 'multrees' option was kept ineffective, and 1000 bootstrap replicates were used to calculate the clade support values. Subsequently, a 50% majority rule consensus tree was generated from the bootstrap replicates. Consistency index (CI) and Retention index (RI) were also calculated in PAUP*. Additionally, Maximum Likelihood (ML) analysis was conducted with RAxML-HPC2 version 7.4.2 (Stamatakis 2006) by generating 1000 ML trees using the random parsimonious tree as the starting tree and using GTRGAMMAI as the DNA substitution model. One thousand bootstrap replicates were used to estimate clade support on the maximum likelihood tree. Finally, we performed Bayesian analysis using MrBayes version 3.2.5 (Ronquist and Huelsenbeck 2003). The best DNA substitution models were selected based on Partition Finder results, and prior probabilities were set to the default values for selected models. Two runs were initiated with random trees, and four chains were included in each run of which three were heated and one was cold. Analysis was carried out for >1 million generations with the temperature set to 0.2 for sufficient swapping between hot and cold chains. Cold chains were sampled after every 10,000 generations and cross diagnosis of two runs was carried out after every 500 generations. Tracer version 1.6 (Rambaut and Drummond 2007) was used to determine the number of generations with consistent marginal probabilities. To compute the consensus Bayesian tree, results from initial 25% generations were discarded owing to their unstable posterior probability values. Fig Tree version 1.4.2 (Rambaut 2007) was used to visualize the phylogenetic trees rooted with their respective outgroups.

Here we report the results of MP, ML, and Bayesian analyses based on combined 1) GenBank extracted nrDNA loci sequences of *C. filaginifolia*, 2) cpDNA loci from *C. filaginifolia* var. *incana* and *C. filaginifolia* var. *linifolia* individuals.

Statistical Analyses

We used sequences downloaded from GenBank representing *C. filaginifolia* specimens from across California to conduct an analysis of molecular variance (AMOVA) to determine haplotype diversity in GENALEX version 6.5 (Peakall and Smouse 2006). The sequences were divided into four groups based on ecoregions and sub-ecoregions of California (McNab et al. 2007). Sequences representing Del Norte, Mendocino, and Marin counties were categorized as a single group from Northern California Coast region. Those from Santa Clara, Santa Cruz, Merced, San Benito and Monterey counties were clustered together representing the Central California Coast and Coast Ranges region. Sequences generated from individuals collected inside Fresno, Kern, Madera, and Mariposa counties were placed under Great Valley and Sierra Nevada region, whereas sequences from Los Angeles, San Bernardino and

San Diego were clustered into Southern California Coast, Mountain and Valley region. A Mantel test was performed with 999 randomized permutations to compare the genetic distances among *C. filaginifolia* individuals with corresponding geographic distances in GENALEX.

RESULTS

Amplification success varied across loci whereby ITS, *rpoC1*, and *ycf5* loci amplified in 95% of the samples; ITS2 amplified in 80% of the samples; *psbA-trnH* and *rbcL* amplified in 60% of the samples (Table 2). Amplicon lengths for each locus were comparable to sequences generated in previous studies using the same primer pairs (Kress et al. 2005; Chen et al. 2010) (Table 2).

Sequence Alignment, Partition, and Divergence

The overall alignment consistency score was 99 for each locus. The combined alignment of nrDNA loci (3'ETS, 5'ETS, and ITS) based on 31 sequences of *C. filaginifolia* consisted of 1884 characters. The best scheme showed only one partition for nrDNA dataset and K80+G DNA evolution model. The cpDNA alignment based on 20 sequences consisted of 2338 characters and the best scheme showed two partitions for this alignment. The first partition consisted of all cpDNA coding loci (*rbcL*, *rpoC1*, and *ycf5*) with F81+G DNA substitution model. The second partition consisted of the non-coding cpDNA gene (*psbA-trnH*), which was placed under HYG+G DNA evolution model. The nrDNA loci containing sequences from across California (3'ETS, 5'ETS and ITS) exhibited higher average divergences (K2P = 0.001–0.01) within *C. filaginifolia* in comparison to nrDNA loci (ITS2) and all cpDNA loci (K2P = 0.0002–0.001) which contained sequences generated from *C. filaginifolia* individuals sampled within San Diego County (Table 2).

Phylogenetic Analyses of nrDNA Loci

Maximum parsimony analysis showed 9 of 1884 characters to be parsimony informative for the ingroup samples. The average base frequencies were A = 0.22055; C = 0.23907; G = 0.26581; T = 0.27457. With the outgroup taxa added, the program retained 939 most parsimonious trees, and 941 tree islands were obtained from 1000 replications. The tree length of most parsimonious trees was 60. The rescaled consistency index and retention index were 0.9593 and 0.9756, respectively, which provide support for divergent evolution of sequences. The consistency index excluding uninformative characters was 0.9583 (Fig. 3a). Maximum likelihood analysis of the partitioned dataset yielded 1000 trees with the maximum likelihood score range of –3057.920 to –3057.928. The best tree was used to compute bipartitions with 1000 bootstrapped replicates, and

TABLE 1. GenBank accession numbers of *Corethrogyne filaginifolia* and outgroup sequences used in this study, generated from chloroplast DNA (*psbA-trnH*, *rbcL*, *rpoC1*, *ycf5*) and nuclear ribosomal DNA (ITS, and ITS2) loci. *Corethrogyne filaginifolia* var. *linifolia* (*C. f.* var. *linifolia*) leaf samples were collected from Torrey Pines State Natural Reserve (TPSR) extension in Del Mar, San Diego County, CA, and Point Loma (PL), San Diego County, CA. *Corethrogyne filaginifolia* var. *incana* (*C. f.* var. *incana*) leaf samples were collected from six different locations on Point Loma peninsula, San Diego County, CA, which included Cabrillo Drive (CabN, CabS), McClelland Road (McC), SPAWAR (Spa), Electron Drive (Elec), and National Park Service (NPS). The sample codes used in this study (i.e., Sample_ID column) include the location or county from where the sample was collected. The blank cells represent unsuccessful locus amplification in samples. All DNA samples are stored in J. Sharma’s lab, Texas Tech University, Lubbock, TX.

Sample_ID	Taxon	Collector	Herbarium	Collection	date	GenBank Accession numbers										
		Number				accession	number	psbA-trnH	rbcL	rpoC1	ycf5	ITS	ITS2	3' ETS	5' ETS	
Cfl_TPSR1	C. f. var. linifolia				19 May 2014				MF135576	MF167337	MF183975	MF183994				
Cfl_TPSR2	C. f. var. linifolia				19 May 2014			MF167349	MF167320	MF167338	MF183976	MF183995				
Cfl_TPSR3	C. f. var. linifolia				19 May 2014			MF167350	MF167321	MF167339	MF183977	MF183996				
Cfl_PL	C. f. var. linifolia				16 May 2014				MF135575	MF167336	MF183978	MF183997				
Cfi_CabN1	C. f. var. incana				20 May 2014			MF167340	MF167310	MF167322	MF183979	MF183998				
Cfi_CabS1	C. f. var. incana				20 May 2014			MF167341	MF167311	MF167323	MF183980	MF183999				
Cfi_CabS2	C. f. var. incana				20 May 2014			MF167342	MF167312	MF167324	MF183981	MF184000				
Cfi_CabS3	C. f. var. incana				20 May 2014			MF167343	MF167313	MF167325	MF183982	MF184001				
Cfi_McC1	C. f. var. incana				20 May 2014				MF135567	MF167329	MF183983					
Cfi_McC2	C. f. var. incana				20 May 2014					MF167330	MF183984					
Cfi_McC3	C. f. var. incana				20 May 2014				MF135568	MF167331	MF183985	MF184002				
Cfi_Elec1	C. f. var. incana				20 May 2014				MF135564	MF167326	MF183986	MF184003				
Cfi_Elec2	C. f. var. incana				20 May 2014				MF135565	MF167327		MF184004				
Cfi_Elec3	C. f. var. incana				20 May 2014			MF167344	MF167315	MF167328	MF183987	MF184005				
Cfi_Spa1	C. f. var. incana				20 May 2014				MF135572		MF183988	MF184006				
Cfi_Spa2	C. f. var. incana				20 May 2014				MF135573	MF167334	MF183989	MF184007				
Cfi_Spa3	C. f. var. incana				20 May 2014			MF167348	MF167319	MF167335	MF183990	MF184008				
Cfi_NPS1	C. f. var. incana				20 May 2014			MF167345	MF167316	MF167331	MF183991	MF184009				
Cfi_NPS2	C. f. var. incana				20 May 2014			MF167346	MF167317	MF135570	MF183992					
Cfi_NPS3	C. f. var. incana				20 May 2014			MF167347	MF167318	MF135571	MF183993					
Bidens_pilosa								GQ435068	GQ436430	GQ435601		GQ434471				
Cf_San_Diego_1	C. filaginifolia	Reiser s.n.	SD 152639								JX680472.1		JX680499.1			
Cf_San_Diego_2	C. filaginifolia	Rebman 9514	SD 164291								JX680473.1		JX680500.1			
Cf_San_Diego_3	C. filaginifolia	Rich 109	SD 153413								JX680474.1		JX680501.1			
Cf_San_Diego_4	C. filaginifolia	Rebman 8252	SD 160694								JX680475.1		JX680502.1			
Cf_San_Diego_5	C. filaginifolia	Rebman 9579	SD 156442								JX680476.1		JX680503.1			
Cf_San_Diego_6	C. filaginifolia	Gregory 1179	SD 160377								JX680477.1		JX680504.1			
Cf_San_Diego_7	C. filaginifolia	Wallace s.n.	JEPS 126512								JX680478.1		JX680505.1			
Cf_Fresno_1	C. filaginifolia	Taylor 717	JEPS 090954								JX680479.1		JX680506.1			
Cf_Kern_1	C. filaginifolia	Markos 158	JEPS 126513								JX680480.1		JX680507.1			
Cf_San_Bernardino_1	C. filaginifolia	Markos 202	JEPS 126515								JX680481.1		JX680508.1			
Cf_San_Bernardino_2																
Cf_San_Monterey_1	C. filaginifolia	Markos 203	JEPS 126516								JX680482.1		JX680509.1			
Cf_Monterey_1	C. filaginifolia	CR 338	JEPS 119402								JX680483.1		JX680510.0			

TABLE 1. CONTINUED

Sample_ID	Taxon	Collector and Collection Number	Herbarium accession number	Collection date	GenBank Accession numbers						
					<i>psbA-trnH</i>	<i>rbcL</i>	<i>rpoC1</i>	<i>ycf5</i>	ITS	ITS2	3' ETS
Cf_Del_Norte_1	<i>C. filaginifolia</i>	<i>Parks & Tracy</i> <i>11496</i>	JEPS 15871					JX680484.1		JX680511.1	
Cf_Mendocino_1	<i>C. filaginifolia</i>	<i>Bacigalupi</i> <i>8993</i>	JEPS 35847					JX680485.1		JX680512.1	
Cf_Marin_1	<i>C. filaginifolia</i>	<i>Robbins</i> 3891	JEPS 22067					JX680486.1		JX680513.1	
Cf_Los_Angles_1	<i>C. filaginifolia</i>	<i>Markos</i> 193	JEPS 126514					JX680487.1		JX680514.5	
Cf_San_Diego_8	<i>C. filaginifolia</i>	<i>Moran</i> 28334	SD 105277					JX680488.1		JX680515.1	
Cf_Mariposa_1	<i>C. filaginifolia</i>	<i>Rose</i> 60125	JEPS 26049					JX680489.1		JX680516.1	
Cf_Santa_Clara_2	<i>C. filaginifolia</i>	<i>Ewan</i> 8119	UC 521768					JX680493.1		JX680520.1	
Cf_Merced_1	<i>C. filaginifolia</i>	<i>Lyon</i> 1572	UC 971972					JX680492.1		JX680519.1	
Cf_San_Benito_1	<i>C. filaginifolia</i>	<i>Lyon</i> 1428	UC 971971					JX680495.1		JX680522.1	
Cf_San_Bernardino_3	<i>C. filaginifolia</i>	<i>Jones</i> 7239	UC 1544816					JX680496.1		JX680523.0	
Cf_Monterey_2	<i>C. filaginifolia</i>	<i>Bacigalupi</i> <i>2692</i>	UC 708668					JX680497.1		JX680524.1	
Cf_San_Diego_10	<i>C. filaginifolia</i>	<i>Reveal</i> 2754	SD 110423					JX680498.1		JX680525.1	
Cf_Mendocino_3	<i>C. filaginifolia</i>	<i>Moore</i> 1018	JEPS 126518					JQ011998.1			
Cf_Santa_Cruz_1	<i>C. filaginifolia</i>	<i>Markos</i> 116	JEPS 88053					AF251593		AF251651	AF322271
Cf_Mendocino_2	<i>C. filaginifolia</i>	<i>Semple</i> 8536	UC 1557828					AF251594		AF251652	AF322272
Cf_Santa_Clara_1	<i>C. filaginifolia</i>	<i>Markos</i> 112	JEPS <i>s.n.</i>					AF251589		AF251647	AF322267
Cf_San_Diego_9	<i>C. filaginifolia</i>	<i>Markos</i> 144	JEPS 88048					AF251590		AF251648	AF322268
Cf_Kern_2	<i>C. filaginifolia</i>	<i>Markos</i> 159	JEPS 126511					AF251591		AF251649	
Cf_Madera_1	<i>C. filaginifolia</i>	<i>Markos</i> 163	JEPS 126510					AF251592		AF251650	AF322269
Lessingia_arachnoidea_1	<i>Lessingia arachnoidea</i>	<i>Markos</i> 210	JEPS 126509					JX680490.1		JX680517.1	
Lessingia_arachnoidea_2	<i>Lessingia arachnoidea</i>	<i>Markos</i> 221	JEPS 126517					JX680491.1		JX680518.1	
Lessingia_glandulifera_1	<i>Lessingia glandulifera</i>	<i>Twisselmann</i> <i>13730</i>	JEPS 54444					JX680494.1		JX680521.1	

TABLE 2. Amplification success of nuclear and chloroplast DNA barcoding loci used for resolving the phylogeny of *Corethrogyne filaginifolia*. Amplicon length represents the range of sequence length across all individuals of *C. filaginifolia*. Nuclear ribosomal DNA (nrDNA) loci 3' ETS, and 5' ETS represent GenBank extracted sequences of *C. filaginifolia*, while the ITS locus represents both GenBank (31), and self-generated (19) sequences. The ITS2 locus of nrDNA and all four chloroplast DNA (cpDNA) loci represent self-generated sequences. Pairwise sequence distances were calculated using Kimura 2-parameter (K2P) DNA substitution model with gamma distribution after pairwise deletion of gaps.

Locus	PCR success (%)	Sequencing success (%)	Amplicon length (bp)	n	Mean K2P distance	Range of K2P distances
nrDNA						
3' ETS	n/a	n/a	n/a	30	0.0031	0.000–0.012
5' ETS	n/a	n/a	n/a	5	0.0154	0.004–0.033
ITS	95	100	659–735	50	0.001	0.000–0.005
ITS2	80	100	436–463	16	0.0003	0.000–0.002
cpDNA						
<i>rbcL</i>	60	100	659–712	12	0.0002	0.000–0.001
<i>rpoC1</i>	95	100	506–510	19	0.0009	0.000–0.004
<i>ycf5</i>	95	100	383–402	19	0.0006	0.000–0.005
<i>psbA-trnH</i>	60	100	390–415	11	0.001	0.000–0.007

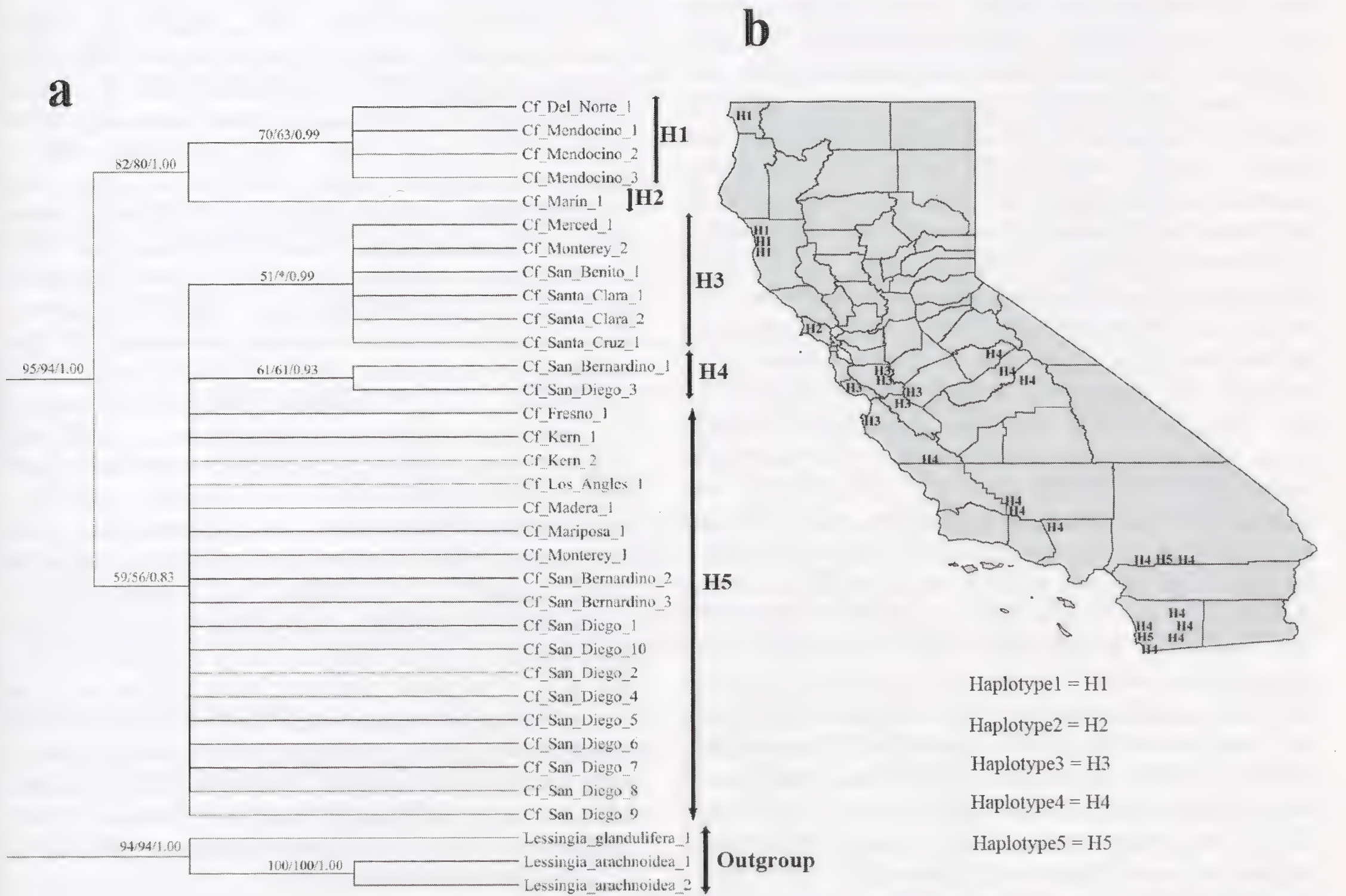


FIG. 3. a) Phylogeny of *Corethrogyne filaginifolia*. The tree was constructed using partitioned dataset that included previously generated GenBank reference sequences from ITS, 3'ETS, and 5'ETS loci from nuclear ribosomal DNA (nrDNA). The clade support values from maximum parsimony, maximum likelihood, and Bayesian analyses are shown for each clade (an * indicates <50% bootstrap support). The potential haplotype clades are indicated with text. b) California map showing the counties and locations of identified haplotypes, each sampling location is not visibly identifiable by text on the map because of the overlap.

TABLE 3. A summary of transitions in ITS and transversions in 3' ETS locus alignments among *Corethrogyne filaginifolia* individuals. The length of the aligned ITS locus was 630 characters and the length of 3' ETS locus was 565 characters. Transitions and transversions are in bold-faced font according to the characterization of different haplotypes. Haplotype5 is used as a reference character state due to its occurrence in majority of the sequences. The character (asterisk*) in haplotype2 differentiates it from haplotype1.

Individuals	Base position in alignment					
	ITS		3' ETS			
	388	619	240	273	325	396
Haplotype1	T	A	C	C	G	G
Haplotype2	T	G*	C	C	G	G
Haplotype3	C	G	G	A	G	T
Haplotype4	C	G	G	A	C	G
Haplotype5	C	G	G	A	G	G

the resultant consensus ML tree was obtained with <50–100% clade support values (Fig. 3a). Additionally, the Bayesian analysis showed convergence of two independent runs after 10 million generations with <0.01 average standard deviation of split frequencies, >200 Expected Sample Size (ESS) and >1 Potential Scale Reduction Factors (PSRF). Marginal likelihood scores of the two runs were very similar (–3081 and –3105) suggesting the search reached convergence. Of the 64 partitions, 30 partitions were informative with >0.1 probability of occurrence across the two runs (Fig. 3a). All three phylogenetic analysis methods were consistent with respect to the relative placement of *C. filaginifolia* individuals (Fig. 3a). The *C. filaginifolia* clade revealed five haplotypes based on ITS and 3'ETS loci (Fig. 3a, Table 3). These haplotypes resulted from two synapomorphies within ITS and four synapomorphies within the 3'ETS. Haplotype5 was chosen as the character reference state for the comparisons of synapomorphies (Table 3). The length of the aligned ITS locus was 630 characters and showed two transitions at the 388th and 619th base position. Haplotype1 and haplotype2 were distinguished based on these two transitions within the ITS locus. The length of the aligned 3'ETS locus was 564 characters, and there were four transversions at 240th, 273th, 325th, and 396th base positions in the alignment. The two transversions at 240th and 273th base positions in 3'ETS locus differentiated haplotype1 and haplotype2 from the other three haplotypes. The single transversions at 325th and 396th base position in 3'ETS locus distinguished haplotype3 and haplotype4, respectively. Additionally, the ITS tree including GenBank and sequences generated from this study grouped all *C. filaginifolia* individuals together to the exclusion of the outgroup samples. Nested within a largely unresolved clade of *C. filaginifolia* samples is a clade including individuals from haplotype1 and haplotype2 which is supported by moderate to high credibility values in

all three phylogenetic analyses (MP = 100%, ML = 63%, BI = 0.88) (Fig. 4). Analysis of Molecular Variance (AMOVA) showed significant differences in nrDNA haplotype diversity of *C. filaginifolia* from four ecoregions ($p = 0.001$). The variation in haplotype diversity within ecoregions was 33%, whereas variation among ecoregions was 67%. The Mantel correlation between genetic and geographic distances was 0.21 ($p = 0.01$).

Phylogenetic Analyses of cpDNA Loci

Maximum Parsimony analysis in PAUP* showed seven out of 2338 DNA characters to be parsimony informative for cpDNA loci of the ingroup samples. The average base frequencies were A = 0.29771; C = 0.18650; G = 0.19061; T = 0.32518. After addition of outgroup taxon, numerous equally parsimonious trees were retained (911), and 915 tree islands were found from 1000 replications. The tree length of the most parsimonious trees was 307. The rescaled consistency index, and retention index were 0.9611 and 0.9643, respectively. The consistency index excluding uninformative characters was 0.9583 (Fig. 5a). One thousand ML trees generated in RAXML had maximum likelihood scores ranging from –4151.323 to –4152.060. The best tree, with a maximum likelihood score of –4151.323, was used to assign clade support values from 1000 bootstraps. Most of the clade support values were less than 50% for the ML analysis (Fig. 5c). In the Bayesian analysis, two independent runs had good convergence with the average standard deviation of split frequencies <0.01 after 2 million generations (ESS > 200, PSRF > 1). The marginal likelihood scores of the two runs were also homogenous (–4255 and –4256). A total 56 partitions were found from which 28 partitions were found to be informative with >0.1 probabilities of occurrence across the two runs (Fig. 5b). None of the analyses showed genetic discrimination between *C. filaginifolia* var. *incana* and *C. filaginifolia* var. *linifolia*. Unlike for the nrDNA data phylogenetic analyses, the topology was different for the each of the three methods. Also, the majority of the clade support values were <50% for ML and MP methods. ML, MP, and Bayesian analyses produced non-monophyletic varieties. In the cpDNA phylogenetic analyses, individual relationships do not reflect geography, nor do the relationships reflect any morphological similarities.

DISCUSSION AND CONCLUSIONS

We report five haplotypes of *C. filaginifolia* based on nrDNA barcoding data generated from specimens collected across California. Of the five haplotypes detected, haplotype1 and haplotype2 together segregated as a separate lineage from the rest (Fig. 3a). Among the five individuals constituting haplotype1 and haplotype2, three were from Mendocino County

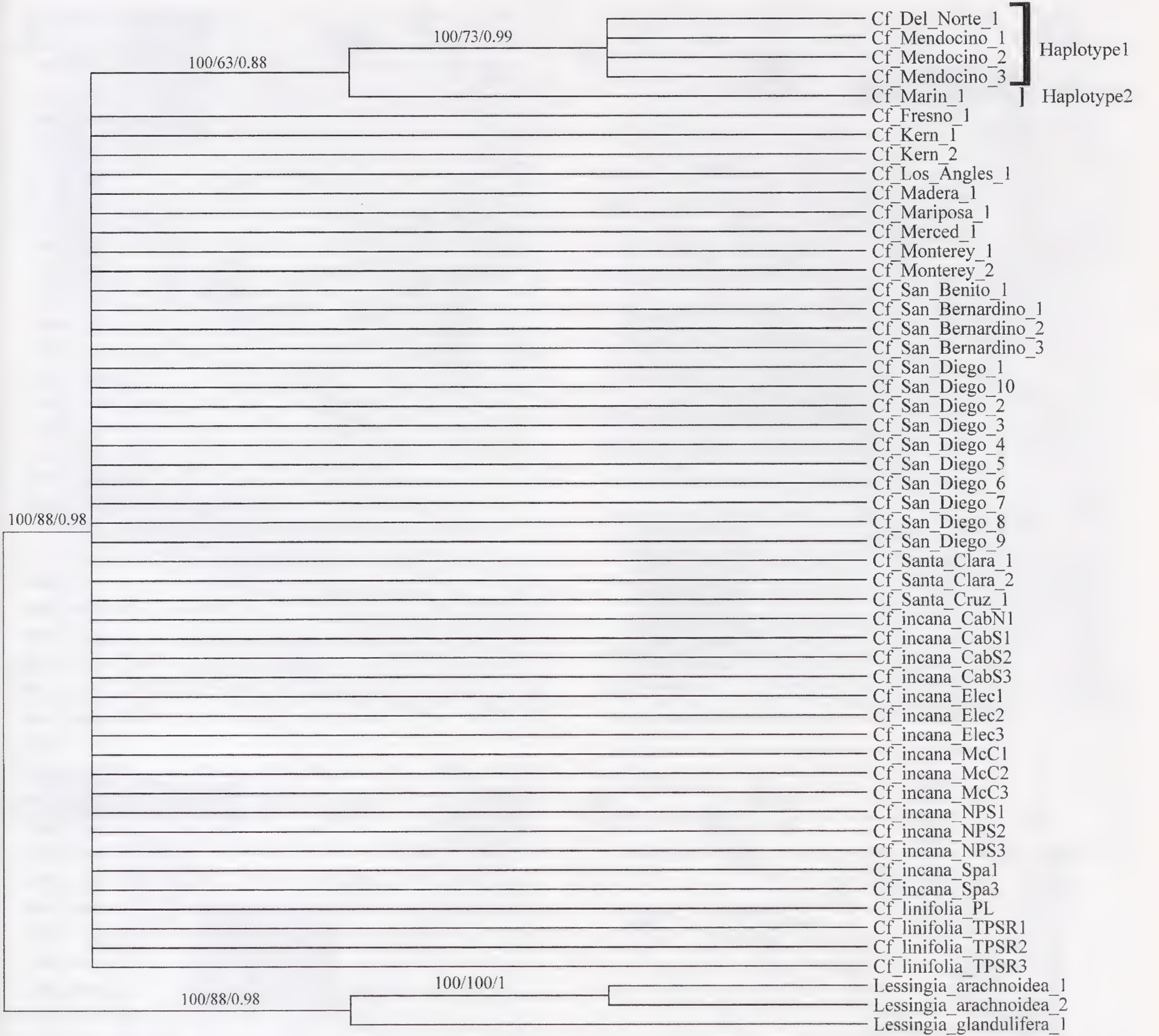


FIG. 4. Phylogeny of *Corethrogyne filaginifolia* based on ITS locus from nuclear ribosomal DNA (nrDNA). The tree was constructed using previously generated ITS GenBank reference sequences, and ITS sequence generated in this study. The clade support values from maximum parsimony, maximum likelihood, and Bayesian analyses are shown. The tree showed clustering of *C. filaginifolia* var. *incana*, *C. filaginifolia* var. *linifolia*, and other GenBank *C. filaginifolia* individuals except the clade of haplotype1 and haplotype2 individuals. *Lessingia arachnoidea* and *L. glandulifera* were used as outgroup species.

(*Bacigalupi* 8993 [JEPS], *Moore* 1018 [JEPS], and *Semple* 8536 [UC]) one from coastal Del Norte County (*Parks & Tracy* 11496 [JEPS]), and one from Marin County (*Robbins* 3891 [JEPS]) (Fig. 3a and b). The populations of *C. filaginifolia* in Mendocino, Del Norte and Marin counties experience similar environmental conditions given their placement within the Northern Coast section of California’s coastal steppe, mixed forest and redwood forest province (McNab et al. 2007). It is possible that these populations constitute a different phylogeographic taxon of *C. filaginifolia*. Moreover, the support values for this clade in the nrDNA cladogram were high (MP = 82%, ML = 80%, Bayesian = 1.00) (Fig. 3a). Within this clade, an individual collected from Marin County (haplotype2) segregated from individ-

uals representing Del Norte and Mendocino counties (haplotype1) due to lack of shared transition at 619th base position in ITS locus (Table 3). However, we are reporting the five individuals belonging to haplotype1 and haplotype2 as a single phylogeographic taxon given their consistent transition and transversions at other positions in ITS and 3’ETS loci (Table 3). Further, the morphology of a specimen collected from Marin County (*Robbins* 3891 [JEPS]) was similar to the vouchers representing Del Norte (*Parks & Tracy* 11496 [JEPS]), and Mendocino counties (*Bacigalupi* 8993 [JEPS], *Moore* 1018 [JEPS], and *Semple* 8536 [UC]). Additional multiple samples of *C. filaginifolia* should be included from the southern limits of North Coast of California to document the genetic and morphological variation

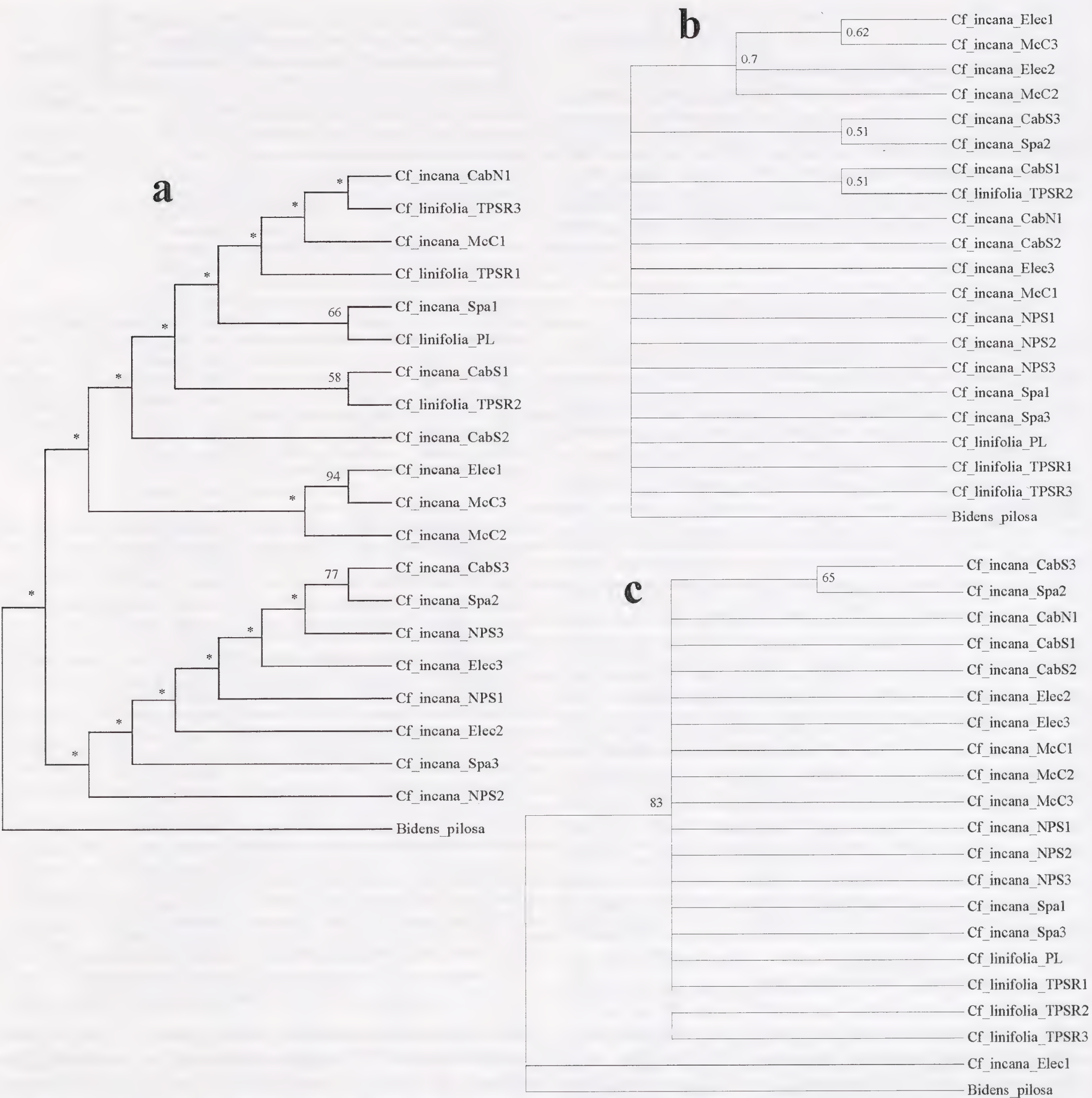


FIG. 5. Phylogeny of *Corethrogyne filaginifolia* based on cpDNA, which included sequences generated in the present study from *psbA-trnH*, *rbcL*, *rpoC1*, and *ycf5* loci with partitioned dataset. The name of each individual is followed by the collection site code shown in Fig. 1 and Table 1. The trees provide topology and clade support values from, a) maximum parsimony (an * indicates <50% bootstrap support), b) Bayesian analyses, and c) maximum likelihood analyses methods. *Bidens pilosa* was added as an outgroup species. The trees showed polytomy of *C. filaginifolia* var. *incana* and *C. filaginifolia* var. *linifolia*.

within this region. The voucher specimens of haplotype1 (*Parks & Tracy 11496* [JEPS], *Bacigalupi 8993* [JEPS], *Moore 1018* [JEPS], and *Semple 8536* [UC]), and haplotype2 (*Robbins 3891* [JEPS]) showed clear morphological distinctions in foliage when compared to other haplotypes (Fig. 6). The leaves of haplotype1 and haplotype2 are spatulate and apically serrate (Fig. 6a), whereas the leaves of haplotypes 3, 4, and 5 are obovate, oblanceolate to linear, and without serrations (Fig. 6b–d). Haplotype3 was identified from specimens from Santa Cruz

(*Markos 116* [JEPS]), Santa Clara (*Ewan 8119* [UC]), San Benito (*Lyon 1428* [UC]), Monterey (*Bacigalupi 2692* [UC]), and Merced (*Lyon 1572* [UC]) counties, which fall under California central coast and coastal ranges ecoregions. The specimens from this region shared only a single transversion, and the clade support values for haplotype3 were low in both MP and ML analyses (51% and <50%, respectively) (Fig. 3a). Thus, we cannot be confident in haplotype3 being a separate phylogeographic taxon. Although, the morphology of specimens in haplotype3 was

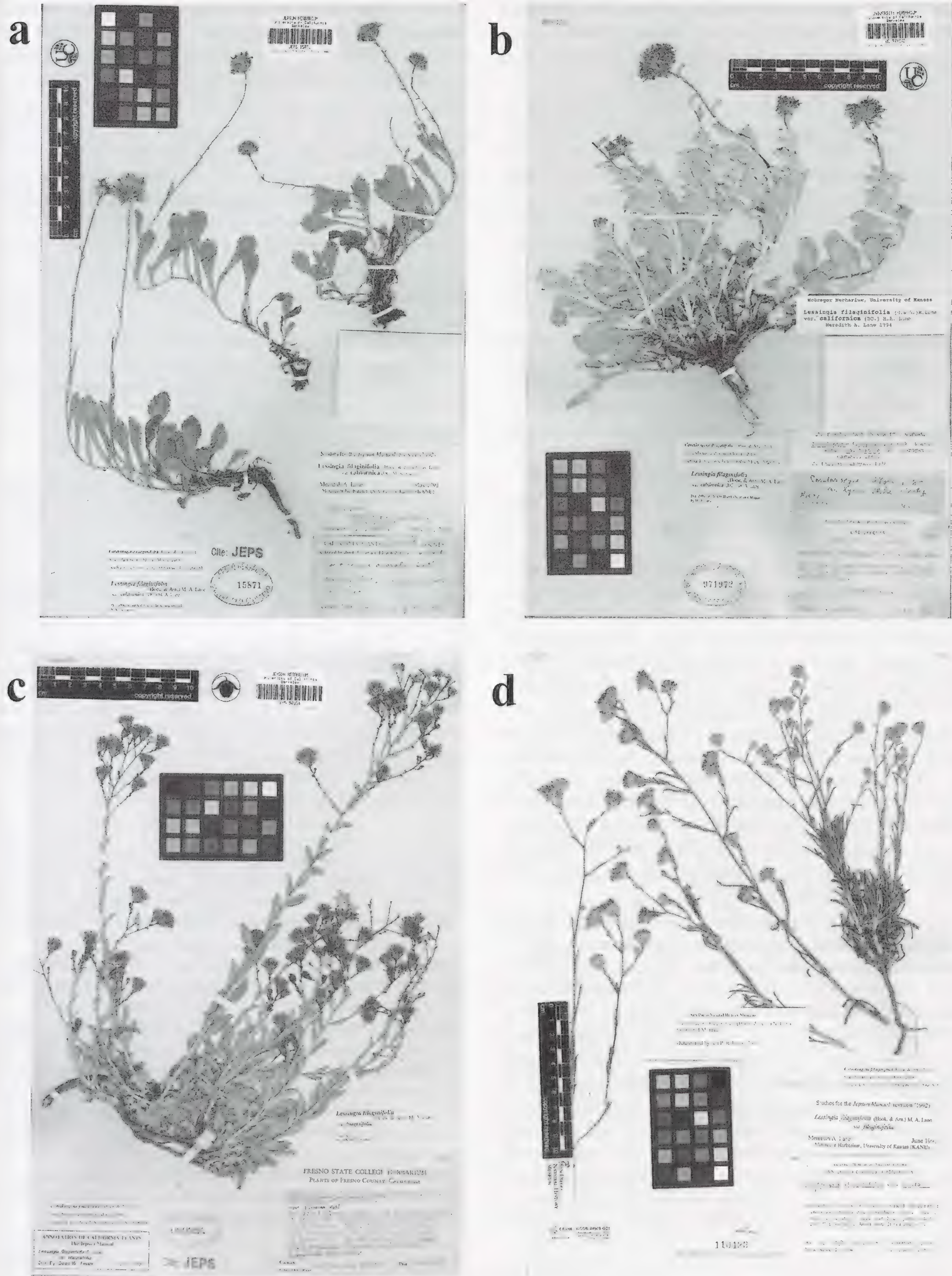


FIG. 6. Morphological variation among herbarium voucher specimens of *Corethrogyne filaginifolia* corresponding to the sequences from GenBank that we used as reference sequences to identify haplotypes. a) JEPS 15871, individual of *C. filaginifolia* from Del Norte County representing haplotype1 and haplotype2. The plant shows primary growth of shoot and spatulate leaves that are apically serrated. b) UC 971972, individual of *C. filaginifolia* from Merced County, a representative of haplotype3. The plant shows secondary growth with outward growth of shoots, and obovate leaves with entire margins. c) JEPS 090954, *C. filaginifolia* individual from Fresno County, a representative of haplotype5. The plants have oblanceolate leaves. d) SD 110423, *C. filaginifolia* var. *linifolia* individual from San Diego County, a representative of haplotype5 with narrow leaves and tomentose involucral bracts.

distinct in terms of plant growth, haplotype3 individuals exhibited more secondary growth along shoots in comparison to haplotype1, which showed mostly primary growth (Fig. 6a and b).

Haplotype4 was represented by two specimens, one from San Diego County (*Rich 109* [SD]), and the other from San Bernardino County (*Markos 202* [JEPS]). This haplotype was characterized by a single transversion in 3'ETS locus, and the clade support values were low in the parsimony and likelihood analyses (MP = 61%, ML = 61%), but high in the Bayesian analysis (BI = 0.93) (Fig. 3a). The representative specimen of haplotype4 from San Diego County (*Rich 109* [SD]) was identified as *C. filaginifolia* var. *linifolia*; whereas, the varietal annotation of the representative individual from San Bernardino County could not be ascertained (*Markos 202* [JEPS]). *Corethrogyne filaginifolia* var. *linifolia* has not been reported outside San Diego County, thus the grouping of *C. filaginifolia* var. *linifolia* individual from San Diego County with *C. filaginifolia* individual from San Bernardino County does not allow for confident inferences about haplotype4. Moreover, individual Cf_San_Diego_10 (*Reveal 2754* [SD]) from haplotype5 was also identified as *C. filaginifolia* var. *linifolia*. Haplotype5 was formed by individuals from Central Coast (*CR 338* [JEPS]), Great Valley and Sierra Nevada (*Taylor 717* [JEPS], *Markos 158* [JEPS], *Rose 60125* [JEPS], *Lyon 1572* [UC], *Markos 159* [JEPS], and *Markos 163* [JEPS]), Southern Coast, and Mountain Valley (*Reiser s.n.* [SD], *Rebman 9514* [SD], *Rebman 8252* [SD], *Rebman 9579* [SD], *Gregory 1179* [SD], *Wallace s.n.* [JEPS], *Markos 203* [JEPS], *Markos 193* [JEPS], *Moran 28334* [SD], *Jones 7239* [UC], and *Reveal 2754* [SD]) sections of California's ecoregions. The polytomy including haplotype5 could not be resolved despite its distinctive morphological variation, especially in San Diego County (Fig. 3a).

We did not detect genetic differences among individuals of *C. filaginifolia* var. *incana* and *C. filaginifolia* var. *linifolia* which were examined with both cpDNA and nrDNA data. The distribution of individuals of the two varieties within nrDNA (Fig. 4) and cpDNA (Fig. 5) cannot be explained with respect to either their provenance or morphological characteristics. This result either implies the occurrence of varying rates of mutation in the two varieties at these loci or the unsuitability of cpDNA and nrDNA loci to accurately explain the infraspecific variation for these two varieties. The discordance between gene trees and morphological distinctions of the two varieties can also be a result of incomplete lineage sorting.

The genetic distinction of northern *C. filaginifolia* individuals (haplotype1 and haplotype2) from the rest of the individuals at nrDNA loci supports the morphometric study of *C. filaginifolia* by Saroyan et al. (2000), and we provide evidence in our study to classify these individuals as a distinct variety. The Mantel correlation ($r^2 = 0.21$, $p = 0.01$) between

genetic and geographic distances also provided evidence that morphological variation might be linked to genetic variation along the latitudinal gradient in *C. filaginifolia*. Although morphometric differences also exist within and between other haplotypes of *C. filaginifolia*, the genetic analyses with universal plant barcoding loci could only provide strong evidence for haplotype1 and haplotype2. We suspect that it might not be possible to fully resolve the infraspecific variation in *C. filaginifolia* using the standard plant barcoding loci. Although higher resolution polymorphic markers such as double digest restriction site associated DNA sequencing (ddRAD-seq) of genomic simple sequence repeats (SSRs), single nucleotide polymorphism (SNPs), and transposable elements are some potential approaches to differentiate the infraspecific variability within *C. filaginifolia*.

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CHARACTERS IN *ARCTOSTAPHYLOS* TAXONOMY

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ABSTRACT

There is value in understanding the past and how it has affected the present. Science focuses on empirical findings, and we know that our prior experiences and those of our predecessors play important roles in determining how we interpret the present. We learn from accomplishments and foibles of predecessors and appreciate the real life experiences we have gone through. In our studies of the genus *Arctostaphylos* Adans. we have been struck by the fascinating stories surrounding taxonomists who have played roles in the development of our current understanding of the group. In addition to providing insights, they sometimes provide humor and lessons on the value of competition versus collaboration. We offer this history of the humans that forged the taxonomy behind *Arctostaphylos* classification in this light.

Key Words: Competition, collaboration, Eastwood, Jepson, manzanita, Roof, Wells

Manzanitas are the largely Californian shrubby genus *Arctostaphylos* Adans. that has long presented a challenge for taxonomists. Unlike other Ericaceae taxa such as *Erica* L., where floral traits differ radically, *Arctostaphylos* are rather homogenous in floral traits but distinguished by vegetative characters including postfire resprouting, leaf shape, inflorescence bracts, glandularity, and hairiness of foliage. We have studied the history of this genus and are here to report that the most fascinating characters are those behind the naming of *Arctostaphylos* taxa. Although this colorful genus has attracted more than two-dozen botanists who have addressed the taxonomy of the group, Alice Eastwood, Willis Jepson, and Phil Wells are perhaps the best known. Many more played a role and in the history of plant taxonomy, and few taxa have attracted a more colorful group of scientists.

One of our earliest introductions to this topic was from the enigmatic Philip V. Wells who had a keen interest in *Arctostaphylos* and a contumacious perspective on other scientists studying this genus. He often spoke of the sharp and sometimes bitter rivalry between Willis Linn Jepson and Alice Eastwood, both of whom in the first half of the 20th century seemed locked in a competition to name new *Arctostaphylos* taxa and provide an appropriate treatment. In the context of this background, it was noted from *California Flora* (Munz and Keck 1959) that a synonym for *A. viscida* Parry was *A. jepsonii* Eastwood. When one of us queried Wells about this incongruity, he explained (to paraphrase) that *A. jepsonii* is a synonym because it was considered a hybrid and Eastwood realized this and published the

name with the recognition that it would never be an acceptable name and, based on rules of botanical nomenclature would bury forever the name of Jepson as a legitimate species of *Arctostaphylos*. Although other taxonomists have acknowledged hearing this, we have no way of vetting the story and it may indeed be apocryphal, but in our minds it is part of the *Arctostaphylos* mythology.

Our objective in this article is to describe some aspects of these human characters. Just as new insights into morphological or genetic characters help us understand the evolution of a genus and relationships with their lineages, so too we think that understanding a little of the personalities of those whose work we depend upon will improve our own interactions. Early on we note that most botanists were quite competitive with one another, yet sometimes collaborated. Our lesson from this is that collaboration may well be an avenue to more rapid insights and understanding. We also suggest that progress in this group arose from two processes, one being simply exploration and description, and the second the origin of literally new systematic characters. We view our discussion not as a sociological statement, but as an observation that progress occurs in multiple ways, but collaboration is frequently more successful as issues become more complicated.

18TH AND EARLY 19TH CENTURIES

The modern era of systematically naming plants dates to Carolus Linnaeus (1707–1778), who was also the first to name a manzanita. He called it *Arbutus*

uva-ursi L., later transferred by Sprengel to Adanson's *Arctostaphylos*. Linnaeus was known as quite a character and, with his new classification system, sometimes offended decent society by drawing parallels between plant flowers and human genitalia, e.g., "[t]he calyx is the bedchamber, the filaments the spermatic vessels, the anthers the testes, the pollen the sperm," (Miller 2002, p. 57) all of which seemed rather scandalous. One clergyman noted "Linnean botany is enough to shock female modesty" (Fara 2003, p. 71).

Most of the Europeans involved in the naming of manzanitas during this period were either explorers like von Humboldt, or recipients of specimens from others who were exploring North America. These herbarium specimens were the basis for new taxa. For example, Swiss botanist de Candolle (1778–1841), without leaving home, classified a number of manzanitas from California in the genus *Andromeda* L., all of which were transferred to *Arctostaphylos* by Lindley (1799–1865) from London College.

Two botanical competitors from Europe were Frederick Pursh (1774–1820) and Thomas Nuttall (1786–1859), both of whom moved from England to North America. Ironically, both were hired sequentially by Benjamin Smith Barton of the University of Pennsylvania who was trying to write a new *Flora of North America* (Pennell 1936). Pursh also was hired by President Thomas Jefferson to work up the collections of the Lewis and Clark expedition, a project that moved him away from Barton's project. Thomas Nuttall was an English botanist who lived much of his life traveling and writing about American plants and was next hired by Barton. Pursh and Nuttall both replicated much of the journeys of Lewis and Clark early on, and explored many other areas later in life. Barton died before completing his work and afterwards both Pursh and Nuttall published independent work that moved North American botany forward: Pursh's *Flora Americae Septentrionalis* (containing *Arctostaphylos tomentosa* [Pursh] Lindl.) (Pursh 1814), and Nuttall's *The Genera of North American Plants...* (Nuttall 1818).

Nuttall for a time also was curator of the botanical garden at Harvard and named *Arctostaphylos* species based on material he had collected in California. Sometimes though he would name species on the skimpiest of material, e.g., *A. pumila* Nuttall was based on just leaf samples (Parry 1887). Ironically, Nuttall complained that Pursh did the same, arguing that he named a species from "merely an imperfect capsule" and because of incidents like that "[t]his unfortunate want of fidelity, prevented me from communicating to Mr. Pursh, many of the plants which now appear in this work." (Nuttall 1818, p. 298). Nuttall also had a keen eye for generic differences and clearly articulated reasons for recognizing *Xylococcus bicolor* Nuttall as distinct from *Arctostaphylos*. Something that even 20th century botanists grappled with.

Asa Gray (1810–1888) succeeded Nuttall at Harvard and named several *Arctostaphylos*, though from material collected by others. Gray's opinion carried more weight with some botanists than others, for example, when he moved *X. bicolor* back into *Arctostaphylos*, Willis Jepson accepted it throughout his career, in contrast to Alice Eastwood who did not. Charles C. Parry (1823–1890) was an Englishman who moved to the U.S. and studied under Asa Gray. He collected extensively as part of the U.S. and Mexican Boundary Survey and published an impressive six new *Arctostaphylos* in one year (Wells 1990). Marcus Jones (1852–1934), who it seemed seldom had a nice thing to say about any botanist, wrote that "Parry was a typical toady, as most men had to be to get a position in the Government service. He was a suave, well-groomed society man with little brains, a great feeder of hot air, who slobbered over the great to keep in their good graces" (Jones 1930, p. 3).

FOCUS ON THE WEST COAST

Willis Linn Jepson

Towards the end of the 19th century, a growing academic community on the West Coast shifted the focus of naming *Arctostaphylos* to local taxonomists. A good starting point is with Edward Lee Greene (1843–1915), who was forced to resign his teaching position from a Baptist College in Illinois (Jercinovic 2005), became an Episcopal missionary and headed west, prolifically collecting and naming new plants along the way. His travels led him to the West Coast and an appointment as rector at St. Mark's Episcopal Church in Berkeley. However, when he started teaching Roman Catholic doctrine to the Episcopalians it caused many to leave his church (Jercinovic 2005), which ended badly in "an amazing little tale involving priestly insubordination, fist-cuffs, locked church doors, filibustering sermons, and the Standing Committee, Chancellor, and Bishop of the diocese" not to mention the State Supreme Court (Ridout 1958, p. 51). By the time he named his first manzanita, *Arctostaphylos patula* Greene in 1891, he had been booted from the Episcopal church and was now a devout Roman Catholic.

Greene named several hundred new species mostly from California, of which a respectable two-thirds have withstood the test of time and are still considered valid (Jercinovic 2005). He was well known in the Berkeley community and was an obvious choice for the first botany professorship at the newly founded University of California. During his tenure there, he had a profound influence on California botany. He was a voluminous writer and, according to Jepson (1918), was a classical scholar whose work deserves high praise for its clear and forceful treatment. As seemed to be the case with early California botanists, he founded his own journals for publishing, including *Pittonia*, and with Jepson's cooperation, *Erythea* (Jepson 1918). How-

ever, not everyone shared Jepson's admiration as illustrated by a letter from the botanical explorer John Coulter to Asa Gray "[w]hat in the world is going to become of us with Greene stirring up synonymy with a pitchfork?... has *Pittonia* No. 3 come to your hands? It reads like the work of a crazy man, at least one lost to all sense of propriety." (Jercinovic 2005, p. 5). However, Greene's character exemplifies many of those in our *Arctostaphylos* story, as Charles E. Bessey wrote in a letter to Greene in 1910 "I have a great leaning towards any man who has something of heresy in him. . . . You have dared to be original and that is what pleasures me immensely" (Jercinovic 2005, p. 7).

In reference to his professor, Jepson wrote, "[h]e must have been, I think a very lonely man. The circumstances of his life served to bring this about. He was given to solitary botanizing" (Jepson 1943, p. 4), words that later seem to describe Jepson himself. Student Jepson was primed to excel in California botany and Greene's belief that understanding plants could only be gained through field studies likely reinforced Jepson's own predilections. Apparently Reverend Greene's anti-Darwinian view had little effect on the growing evolutionist Jepson, although conflicts did arise. Conflicts also with the University of California led to Greene's resignation in 1895, and he took his herbarium and moved east, ultimately spending most of the last 11 yr of his life at the Smithsonian Institute in Washington, D.C. (Jercinovic 2005).

Willis Linn Jepson (1867–1946) is one of just a handful of California *Arctostaphylos* taxonomists, a son of pioneer parents who moved by covered wagon from Missouri to California (Figure 1). Born near the present day community of Vacaville (Solano Co.), he grew up with a marked affection for the natural landscape around him. He graduated from the University of California in 1889, and in 1891, as a new graduate student, demonstrated an extraordinary appetite for botany and the natural world. He was one of the signatories incorporating the Sierra Club in 1892 and played a key role in the creation of the Chamisso Botanical Club (named for the French/German botanist, poet and world traveler, authority for *Eschscholzia californica* Cham. and the inspiration for the genus *Camissonia* Link). This latter organization potentially played a significant role in Jepson's career development as Ertter noted "Different members staked out territories, in which trespassing by rivals was discouraged, . . ." (2000, p. 243). This is a theme that reverberates throughout the history of *Arctostaphylos*. Indeed, it appears that competition with LeRoy Abrams (who later published the four volume *Illustrated Flora of the Pacific States*, Abrams and Ferris 1923) from Stanford University compelled Jepson to found, in 1913, the California Botanical Society and "advance his position of leadership in the botany of the state" (Ewan 1987, p. 13).



FIG. 1. Willis Linn Jepson, Professor of Botany at the University of California (Image courtesy of the University and Jepson Herbaria Archives, University of California, Berkeley).

Jepson's affection for the state is illustrated by his early graduate student goal of writing a flora of California. This magnum opus was to be published in several volumes as they were completed, the first coming out in 1909. Marcus Jones (1910) was highly critical of this project, and of Jepson in general, however, Jones was an equal opportunity offender as he was highly critical of most western US botanists. Philip Munz, for example, described Jones' *Contributions to Western Botany*, as being "marked by its cutting criticism of almost all contemporaries" (Munz and Keck 1959, p. 1563). With respect to Jepson's flora project, Jones wrote "[i]t is the judgment of the writer wrong to publish a work in this way for many people unable to buy will subscribe for without counting the final cost and it will work great hardship on them (Jones 1910, p. 71)." He then proceeds to criticize many of the treatments in this first volume, and blasts Jepson for his poor Latin descriptions.

Jones went on to comment "[t]he writer hopes that Mr. Jepson may successfully complete the herculean task he has assumed, and that before it is too late he may modify his methods and improve the quality of

his work and let others labor alongside of him without friction. The work can never become a school manual because of its bulk and cost. There will be a clear field for Prof. Hall or some other botanist to supply that crying need of a handy and compact school book on Californian botany (Jones 1910, p. 75)." We wonder if, despite the critical nature of this comment, it may not have been the impetus for Jepson interrupting his *Flora of California* project to later write his more compact, single volume of *A Manual of the Flowering Plants of California* (Jepson 1925)?

Without question, Jepson was an outstanding field botanist, with an appetite for collecting and a keen eye for ecological patterns and new variants, skills perhaps more keenly developed than his taxonomic astuteness with *Arctostaphylos*. As a graduate student, he and Professor Greene started the journal *Erythea*, and in the first volume he published his first species of *Arctostaphylos*, *A. elegans* Jepson (1895). However, in the long run, Jepson did not have great success with naming of *Arctostaphylos*. Although he published several taxa, which apparently passed immediate peer review, as with science in general, the real peer-review system is how the scientific community accepts the findings in subsequent years and decades.

Arctostaphylos is largely a chaparral genus and Jepson wrote one of the first papers describing California chaparral (Jepson 1896). However, in this paper he mistakenly indicated *A. glauca* Lindl. was found in the Sierra Nevada, an error we bring up only because it suggests he didn't have sufficient interest at the time in the genus to follow what other botanists were writing about manzanitas; five years earlier C. C. Parry made clear that the very distinct *A. glauca* with large coalesced stones was restricted to the coast ranges, and the Sierra Nevada plants with much smaller separable stones were *A. viscida* Parry (Parry 1887). By the time Jepson published his first book based on his Ph.D. dissertation, *A Flora of Western Middle California* (Jepson 1901), he also made this mistake and even in the second edition (Jepson 1911) he was still mistakenly calling the Sierra Nevada plants *A. glauca*. Considering that he made countless trips to the Sierra Nevada during that first decade of the 1900s (Beidleman 2000), suggests that Jepson was slow in developing an interest in this genus. This is also suggested by the fact that his one paragraph description of *A. elegans* in 1895 (Jepson 1895) was followed by a 21-year hiatus until his next publication on *Arctostaphylos*.

In contrast to his taxonomic perspectives, when it comes to *Arctostaphylos*, Jepson's lasting contribution was his ecological and evolutionary astuteness, in particular the recognition of crown sprouting in some manzanita species. This was an ecological trait previously overlooked by all prior *Arctostaphylos* investigators. However, even more profound was his recognition that several *Arctostaphylos* taxa lacked resprouting ability and concentrated seedling recruit-

ment to a single pulse in the first postfire year. The importance of these observations is illustrated by the fact that in 1916 when Jepson founded the journal *Madroño*, he chose for the lead off article "Regeneration in Manzanita" (Jepson 1916). Later, in biological notes in his treatment in *A Flora of California* (1939), Jepson contended that the non-resprouting species that delayed reproduction to the postfire environment represented true "fire-type shrubs." This represents the first suggestion in the literature of an adaptation evolved in response to fire, not just in chaparral but also for any ecosystem. In some respects, this was an idea before its time as it seemed to capture very little attention until 30 yr later when Phil Wells published his classical paper on chaparral evolutionary strategies (Wells 1969).

With respect to *Arctostaphylos* nomenclature, Jepson had less impressive successes and some inexplicable failures. Perhaps the most perplexing mistake Jepson made is related to a localized endemic manzanita in coastal San Diego County. Just as Einstein's cosmological constant was self-acknowledged as the biggest blunder of his career, perhaps Jepson's biggest blunder was his flip-flopping on the naming of this unique taxon. The story begins in the late 1890s with the report of a new manzanita, *A. glandulosa* Eastwood (Eastwood 1897). In Jepson's first treatment of *Arctostaphylos* (Jepson 1922) a new, but very poorly defined variety was published, *A. glandulosa* var. *crassifolia* Jepson. However, later Jepson (1925) made a new combination, *A. tomentosa* var. *crassifolia* (Jepson) Jepson but much later (Jepson 1939) he protested that his original placement of this variety under *A. glandulosa* was an example of *lapsus calamitosus typographicus* (i.e., a calamitous typographical error). However, based on distinctly different leaf anatomies between *A. tomentosa* and *A. glandulosa*, we now know this San Diego taxon is appropriately placed in *A. glandulosa* and Jepson's real *lapsus calamitosus* was reversing himself in 1925.

Jepson's initial taxonomic error in this regard reminds us of the importance of distinctive morphological characters to taxonomy in this challenging genus, such as the presence of stomata restricted to the lower leaf surface in some species but equally distributed on abaxial and adaxial leaf surfaces in others. Early on, Jepson apparently failed to recognize the taxonomic significance of bifacial (*A. tomentosa*) versus isofacial (*A. glandulosa*) leaves. However, his returning the local San Diego taxon to *A. tomentosa* and writing off the original treatment as a typographical error (Jepson 1939) was purely a lapse of attention to details. In his 1939 volume he did recognize this stomatal feature, but never applied it to *Arctostaphylos glandulosa* subsp. *crassifolia*, which he described as a subspecies of *A. tomentosa*. The concept of two different leaf types was beginning to be more widely appreciated at this time (e.g., Adams 1940), and Howell (1945) produced a review

of these two leaf types in *Arctostaphylos* and stressed their taxonomic value.

A similar scenario occurred with his publication of *A. elegans* Jepson, which was followed a couple years later with a note in *Erythea* "[t]his form, I now decide, is to be referred to *A. manzanita* Parry. I do not regard it as even worthy of a varietal name and so make record for the benefit of monographers and others. The name was published in this journal for January, 1893. (Vol. i. p. 15)" (Jepson 1895). However, later Jepson reversed himself once again and included *A. elegans* in Jepson (1922, p. 81) stating, "The note in *Erythea*, 3:178, was an inadvertence." Later, Benson (1940) formally treated this taxon as a variety of *A. manzanita*.

Another unsuccessful taxonomic revision by Jepson appears to have arisen due to his distaste for C. Hart Merriam's (of Life Zone fame, Merriam 1898) naming of *A. mewukka* Merriam and *A. nissenana* Merriam, both species recognized in honor of Native American tribes in California where these species were geographically situated. Regarding *A. mewukka*, Jepson (1922, p. 83) noted, "[t]he specific name, borrowed from the Miwok tribe, seems barbarous. Dr. Merriam has cultivated Indian lore and tribal habits so long that *meukka* [sic] to him is probably as pleasing as the lucent phrases of the *Ars Poetica* to the ear of Horace." Perhaps as a consequence, Jepson (1922, p. 83) felt Merriam's description of *A. mewukka* was "insufficiently described to be placed with certainty..." and so Jepson erected a new name, *A. pastillosa* Jeps., which has never been accepted by other *Arctostaphylos* taxonomists. However, by 1939 he resurrected Merriam's *A. mewukka* and *A. nissenana* and dissolved *A. pastillosa* (Jepson 1939).

One of Jepson's last students was J.E. Adams (1903–1981), who published his systematic study of *Arctostaphylos* a year after Jepson's 1939 treatment (Adams 1940). Although Adams' treatment was broadly similar to his professor's, it was much closer to contemporary thinking about the genus, including rejection of Jepson's claim of *lapsus calamitosus typographicus* vis a vis *A. glandulosa* var. *crassifolia*. He also rejected Jepson's persistence in subsuming *Xylococcus bicolor* as an *Arctostaphylos* as done by Gray. Adams clearly understood the fruit characteristics that separated *Xylococcus* from *Arctostaphylos* as described by Nuttall in his original description (Nuttall 1843). In addition, for the first time in *Arctostaphylos* taxonomy, Adams included subspecies in addition to varieties to cover subspecific variation.

One of the more unfortunate parts of the Jepson *Arctostaphylos* story is that his former student, A. Everett Wieslander (1890–1992), accused him of essentially pilfering three new *Arctostaphylos* species and publishing them without consultation (Wieslander and Schreiber 1939). An opening footnote to that paper read:

"While this article was in press, Dr. W. L. Jepson, to whom a copy of the manuscript was submitted for

criticism on November 17, 1938, published two papers, "Embryonic Panicles in *Arctostaphylos*" (*Erythea* 8:97. December 22, 1938)... "Three New Californian *Arctostaphyli*" (l.c. 8:99). The use of my name as co-author of *A. pilosula* Jepson & Wieslander, *A. rudis* Jepson & Wieslander, and *A. silvicola* Jepson & Wieslander was unauthorized and without my knowledge or consent. A. E. Wieslander."

According to Wieslander the proofs of this paper, which were to be published in *Madroño*, were sent to Jepson, despite a warning by the editor Herbert Mason to not share them with him (Lage and Wieslander 1985). It appears that in order to have his name attached to these species, Jepson resurrected the journal *Erythea*, which he had co-founded and was editor of but had last been published in 1922. In December 1938, he published "Three New *Arctostaphyli*" in which he named these taxa (Jepson 1938a), but did include "Jepson and Wieslander" as authorities for the new species indicating Wieslander's role in their discovery. According to Wieslander (Lage and Wieslander 1985), Jepson had never seen or designated a type specimen of the three taxa.

In Wieslander and Schreiber (1939), the authors republished these three species as "sp. emend" providing more complete descriptions and designating type specimens. Given these facts, the most parsimonious conclusion is that Jepson was trying to preempt the naming of these three manzanita species so that he would have his name associated with them. Up to this point, as suggested above, Jepson had recognized very few species of *Arctostaphylos*. For someone who related so strongly to this genus, perhaps his lack of success in the discovery of new taxa, and the opportunity to more firmly attach his name to *Arctostaphylos* nomenclature, were important drivers behind this seemingly desperate attempt to "scoop" his former student in naming the three Wieslander and Schreiber taxa. An alternative view is that Jepson for some reason (lost in the mist of time) believed that his name should be associated with these three taxa and some evidence for this hypothesis is that in the Wieslander and Schreiber (1939) paper there were two species, *A. morroensis* Wieslander & Schreiber and *A. otayensis* Wieslander & Schreiber, that Jepson did not name, suggesting he had his reasons for believing he was a rightful authority for the three species he did publish. We will never know.

When Wieslander was put in charge of the Forest Survey for the Vegetation Type Mapping project in 1926, he was requested to determine the sprouting capacity of each species of the chaparral because of its practical bearing on the construction and maintenance of firebreaks (now known as fuel breaks), "sprouting" or "nonsprouting" became the first point of observation in the field. However, since this trait is generally most evident after cutting or burning, he noted "In an effort to clear up this difficulty we were rewarded by the observation that the burl is not a reaction of the species to mutilation by fire or

cutting, as many have understood it to be, but a normal structure which appears early in the life of the seedling... even where fires have never occurred" (Wieslander and Schreider 1939, p. 39). This detail was unknown to Jepson when he first elaborated on resprouting (Jepson 1916, p. 3); with respect to *A. glandulosa* "After the stems are fire-killed, young plants *begin* to form a root-crown, which becomes turnip-shaped or globose," suggesting this might be a coppicing effect. Wieslander and Schreiber (1939) set the record straight by presenting a photograph showing the early stages of burl formation in *A. glandulosa* seedlings and saplings, thus demonstrating that burl formation was an ontogenetic trait initiated early in development. However, how much of this was Wieslander's own observations was unclear, since by then Jepson had incorporated this thinking into his own writings (Jepson 1939).

In the Wieslander and Schreiber (1939) footnote, there was also mention of a second paper Jepson hastily published on embryonic panicles (Jepson 1938b). Wieslander and Schreiber were planning on introducing observations regarding panicle development in their 1939 paper and point out that panicles were of taxonomic value in sorting out *Arctostaphylos* species because "[b]otanists had never used them to identify the manzanitas" (Lage and Wieslander 1985, p. 174). In the year prior to publication, Wieslander stood up after a Berkeley botany department seminar and stated how his field assistant "Mr. Jensen had found that the panicles had characteristics that were very helpful in identifying different species of manzanita, and they were present on the plants longer than the flowers and fruits. Then I [Wieslander] got a four page-letter written by Professor Jepson. He said he had never been so mortified in all his life. He said, "I discovered the differences in embryonic panicles myself. You embarrassed me... There was something wrong with him, I think" (Lage and Wieslander 1985, p. 175). This is consistent with Herbert Mason's (1947, p. 62) description of Jepson "Almost every incident of his stormy life was a drama, a fact that was always in his consciousness even to the point of histrionics."

In his 1938 embryonic panicle paper, Jepson contended that he had known and understood the taxonomic importance of these structures since 1915, and went on to describe many of the differences in these structures that set species apart. A similar discussion is given in the opening to his treatment of *Arctostaphylos* the following year (Jepson 1939), however, nowhere in the treatment does he describe embryonic panicles of the different species or use them to separate the taxa, so it would appear this discussion of embryonic panicles was an after-thought, added hastily before publication. Today these traits are critically important to distinguishing among *Arctostaphylos* taxa, however, Jepson's descriptive term for this trait did not endure the test of time as today we follow the suggestion of Mr. Jensen in calling them "nascent inflorescences."

As a teacher, Jepson was an exponent of the theory of self-reliance and personal experience. Apparently this worked for him in his career and he viewed it as the correct path for all students. Although there are numerous reports of positive interactions with a wide variety of people Jepson encountered throughout his travels (Ertter 2000), at home he was often an irascible colleague or professor. One of his students, Herbert L. Mason (1896–1994), contended that Jepson was very protective of his time and suggested this was one reason why Jepson never married (Mason 1947). However, he was an early suitor of Alice Eastwood, but apparently she never encouraged it and Moore (1996) suggested she never married either for similar reasons. Herbert Mason was a student who graduated under Jepson in 1932 and in his somewhat over the top obituary Mason (1947, p. 62) described the trials of being a Jepson student; Jepson "almost never took a student into the field with him and students could not knock on his perpetually closed office door, but rather they needed to send a letter requesting an appointment."

Finally, in contrast to many who have worked in *Arctostaphylos*, Jepson appeared to look more for similarities among taxa rather than differences. Consequently, Jepson tended to "lump" taxa together as varieties of more wide-ranging species (e.g., lumping several taxa as varieties of *A. andersonii* Gray, *A. montana* Eastw. into *A. pungens* Kunth, *A. franciscana* Eastw into *A. hookeri* G. Don, and *A. virgata* Eastw. as a variety of *A. glandulosa* in Jepson's 1922 treatment). This philosophy possibly influenced his taxonomic approach and resulted in the relatively few species he named. It was also adopted to a degree by Adams (1940) and later by Munz and Keck (1959) in his treatment in *A California Flora*. This taxonomic approach was also the polar opposite from Alice Eastwood, and later manzanita taxonomists (e.g., Phil Wells), who tended to fall on the "splitter" side. With this contrast in mind, we turn our discussion to another one of the most remarkable characters in *Arctostaphylos* lore, Eastwood.

Alice Eastwood

Alice Eastwood (1859–1953) was a self-taught botanist, and although high school valedictorian, she never pursued a college education. At an early age her mother died and her father had financial difficulties so she grew up during her formidable early teenage years in a convent near Toronto (Dakin 1953). Her first interests in botany were cultivated by the convent priests and relatives who were interested in experimental horticulture. She eventually moved to Colorado to live once again with her father, but he was not terribly successful at business and so she had to work several jobs to earn her way through high school. The freedom of exploring the Rocky Mountain landscape instilled in her an interest in native

plants and she acquired a significant personal herbarium.

In 1890–1891, as an amateur botanist she made a plant collecting trip to California and visited Katharine Brandegee and her husband at the California Academy of Sciences in San Francisco (Crosswhite and Crosswhite 1985). The Brandegees were impressed with Eastwood's expertise in botany and ultimately offered her a position at the California Academy of Sciences. Eventually, in 1894 Eastwood took over as head of the botany department, a position she held until retirement in 1949. Her early perambulations around Mount Tamalpais in Marin County yielded three important new manzanita species: *A. glandulosa*, *A. canescens*, and *A. montana* (Eastwood 1897). Her fascination with manzanitas was thus launched and her outdoor explorations were likely enhanced by being one of the few women ever admitted to the Cross Country Boys Club (Thompson 2016). Also early in her career at the academy, she gained notoriety nationally for her heroic saving of nearly 1500 type specimens when most of the collection was otherwise destroyed by fire following the 1906 San Francisco earthquake. This feat was greatly facilitated by the fact that, contrary to the convention at the time, she had stored all type specimens separately from the main collection, allowing her easy access during fires (Moore 1996).

Eastwood apparently guarded her time and avoided getting married because she wished to pursue her career without hindrances (Moore 1996), but this was not uncommon for the time as nearly three quarters of all 19th century female botanists remained single throughout their careers (Rudolph 1982). However, she did come close to marrying on a couple of occasions, while she was still in Colorado, the sudden death of her intended, had encouraged the move to accept Brandegee's job offer at the Cal Academy (Moore 1996). During Sierra Club outings she became acquainted with geologist Grove Karl Gilbert (1843–1918) and their relationship matured so that by 1918 they decided to marry (Pyne 1980). Gilbert though was reluctant because as he put it, "Alice and I have been lovers for years but for a long time I would not propose marriage because it seemed like asking her to give up a life that satisfied her to become the nurse of my broken health" (Pyne 1980, p. 262). However, his health improved and they planned on marrying, but he died that year of a heart attack at the age of 75.

Eastwood was well poised to deal with the complicated genus *Arctostaphylos*. Particularly *apropos* to this genus was a letter in which she regarded species as a human abstraction imposed on nature. "While I do not hope to straighten out a genus which is not straight in Nature I hope to make the relationships a little clearer than they are now" (Moore 1996, p. 45). Throughout her life she was a risk taker, spending years at a time traveling to herbaria in the east and overseas, hoping her Academy job would still be there when she returned.

As she described it "When one has little one can afford to take risks" (Moore 1996, p. 175). Privately she criticized many herbarium taxonomists on their lack of field knowledge of the species with which they were dealing (Moore 1996). We would expect that she would have held Jepson in high regard in this respect.

Perhaps the interactions between Alice Eastwood and Willis Linn Jepson were to some degree preordained through the negative relationship of their mentors. Alice Eastwood's career was strongly influenced by Brandegee while Edward L. Greene played a significant role in Willis Linn Jepson's early career. Brandegee, however, despised Greene for a number of reasons and her vitriolic prose about his deficiencies were extreme. Part of Brandegee's dislike of Greene was that he was an outspoken critic of Darwin and she published comments such as "This kind of botany was taught, probably in the Middle Ages to which Mr. Greene properly belongs" (Carter 2011, p. 200).

The earliest communication we can find between Eastwood and Jepson was a cordial letter in response to Jepson sending his published dissertation to her for review. She congratulated him on his accomplishment and noted, "Of course we differ in opinion on some points but that is to be expected if both are honest and independent" (Eastwood 1901, no pagination). After that there is relatively little correspondence between the two and Moore (1996) suggested that Jepson and Eastwood did not particularly get along. Nonetheless, although not close colleagues, she maintained a cordial relationship with Jepson in their early years (Daniel 2008).

It seems likely that her professional interest in *Arctostaphylos* taxonomy may have been irritating to Jepson, and presumably *vice versa*. Indeed, Eastwood over her career had substantially greater success in discovering and naming *Arctostaphylos* species. Eastwood had a careful eye with a keen appreciation for subtle differences among taxa. Over her long career, she would name the most manzanita species of all other manzanita taxonomists (30), fourteen of which would ultimately stand the test of time (Parker et al. 2012). By contrast, as discussed above, Jepson described only a few *Arctostaphylos* taxa that have stood the test of time.

Despite Alice Eastwood's keen eye for detail, not all of her decisions have weathered the long-term peer review process, e.g., her separating four species into the genus *Schizococcus* Eastw. (Eastwood 1934, 1937). This separation was largely based on their fruit pulp, which shattered prior to dispersal. However, this characteristic of dehiscent fruits has been reported from other species in the genus *Arctostaphylos* (Keeley 1995), and for this and other reasons (e.g., Howell 1955) few botanists recognize *Schizococcus* and later, molecular genetic data confirmed that these four species are solidly embedded within *Arctostaphylos* (Hileman et al. 2001); indeed one *Schizococcus* species (*A. nissenana*) is in a

different deep lineage than the other three *Schizococcus* (Boykin et al. 2005, Wahlert et al. 2009).

Eastwood is often quoted as having said “I count my age by friends, not years—and I am rich in friends.” She took special delight in honoring friends by using their names for the specific epithet of new species, and over 100 species were so named (Cantelow and Cantelow 1957, p. 83). One of her closest friends and colleagues was John Thomas Howell who worked alongside her for many years and eventually took over the reins of the herbarium at the California Academy of Sciences when Eastwood died. J.T. Howell (1903–1994) was a native Californian born in Merced and an avid botanist by the time he entered high school. He studied under Jepson but notes he was rather underwhelmed by Jepson’s preference for research over teaching (McHoul 1975), and he later received a master’s degree from UC Berkeley in 1927. He was the first resident botanist at the Rancho Santa Ana Botanic Garden, back when it actually was in Santa Ana Canyon, as opposed to residing in Claremont, as is the case today. However, he and the garden founder did not get along well and when Eastwood offered Howell a position as her assistant at the Academy, he reportedly said “Miss Eastwood, I have to tell you I’ve just been fired from a job as resident botanist by Mrs. Susan Bryant at the Rancho Santa Ana Botanic Garden;” to which Eastwood replied, “Mr. Howell, coming from Mrs. Bryant I consider that a recommendation” (Smith 1989, p. 13).

Howell’s and Eastwood’s careers at the Academy overlapped by more than 20 yr, although Howell was usually viewed as Alice’s assistant. Moore (1996) suggested that this was illustrative of Eastwood’s safeguarding her status in the Academy. More than likely the fact that Eastwood was 70 when she hired 26 yr-old Howell (Figure 2) had more to do with their dynamic than professional jealousy. Certainly nothing in the writings of Howell suggested any level of resentment, but when it came to *Arctostaphylos*, both worked rather independently. Ultimately, Howell named only one species, *A. edmundsii*, a coastal endemic from the Big Sur region. This was in 1952, a year before Ms. Eastwood’s death in 1953. Considering their different talents, he with his keen observations on stomatal pattern distribution (Howell 1945) and she with her trained eye for slight taxonomic distinctions among *Arctostaphylos* taxa, suggests that a strong collaboration between these two may have proved synergistic.

One of the significant collaborative efforts between Eastwood and Howell was the creation of the journal *Leaflets of Western Botany* in 1932, apparently for the purpose of expediting their publications of new species. According to Moore (1996), Eastwood started the journal since previous outlets had been closed. The Academy’s official scientific journal was being popularized by the director and would not publish descriptions of new species in Latin. Plus, Eastwood intimated that both she and Howell were



FIG. 2. Alice Eastwood and John Tomas Howell, Washington state, 1936 (with permission from the Anne T. Kent California Room).

frozen out of publication in *Madroño* due to a disagreement with Jepson. Thus, after *Leaflets of Western Botany* was founded in 1932, Eastwood went on a proverbial tear, describing 18 new species of *Arctostaphylos* in 1933 and 1934. Of these, five are still recognized as species today, and seven are recognized at the subspecific level.

This possible conflict with Jepson, and her contention that she was being blocked from publishing in *Madroño*, is consistent with the story Philip Wells relayed in the introduction above, particularly since *A. jepsonii* Eastwood was published following more than two decades of silence on the manzanita front (Eastwood 1934). Perhaps Eastwood and Jepson were not on good terms by this time; Jepson’s *Arctostaphylos* treatment in 1922 omitted several taxa previously recognized by Eastwood and possibly this contributed to Eastwood to independently publish her own revision of the genus (Eastwood 1934). The tension between Jepson and Eastwood was later underscored by the *Arctostaphylos* treatment by Jepson in his *A Flora of California* (Jepson 1939), in which several taxa described by Eastwood in the 1930’s were not recognized. Nonetheless, despite her age and these obstacles, Eastwood published another six species during the war years

(1942–1945), of which one, *A. australis*, expanded her sphere of influence to Baja California. Another, seemingly good taxon, *A. cushingiana* was ignored by Wells, but now recognized as *A. glandulosa* subsp. *cushingiana* (Keeley et al. 2007).

POST-WORLD WAR II

Philip A. Munz (1892–1974), who began his career in 1946 as a botanist at Rancho Santa Ana Botanic Garden (Munz 1947), brought *Arctostaphylos* treatments up to date with *A California Flora* (Munz and Keck 1959). This treatment was the first to replace Jepson's crown-sprouting description with the term basal burl, which persists to the present in manzanita keys (although the term was apparently first used by Dobzhansky 1953). Like Adams (1940), Munz mixed both varieties and subspecies designations, made about a half dozen new subspecific combinations and named the new variety of *A. glandulosa* var. *adamsii* Munz in Adams's honor, describing him as author of a notable study of the genus *Arctostaphylos* (Munz 1958). As noted by Wells (2000), Munz (1958) also added the new species of Wieslander and Schreiber (1939). He additionally elevated some of the Eastwood species that had previously been submerged by Jepson and colleagues.

A new surge of manzanita mania was brought about by Jim Roof, sometimes known as the cantankerous curmudgeon of Tilden Park (Edwards 1999). James B. Roof (1910–1983) was as eccentric as any of the *Arctostaphylos* characters in this story. He was founding director of the Regional Parks Botanical Garden in the East Bay of San Francisco and ran the garden from 1934–1974. During that time, he was editor of the parks botanical journal *Four Seasons*, which he utilized as his primary mechanism for publishing *Arctostaphylos* names and observations - a notable tradition among his immediate *Arctostaphylos* predecessors, several of whom had their own institutional journal for publishing. He was also well known for interrupting the bulldozers leveling Laurel Hill Cemetery in San Francisco, the type locality for *A. franciscana*, and engaging them to help salvage plants that he then moved to the garden in Tilden to conserve this rare species. Roof rightly perceived the conservation implications of *Arctostaphylos* endemism and pursued them vigorously. Despite being a bachelor and living much of his adult life in a one room shack at the garden, he also had an eye for the ladies and raised eyebrows with his repeated use of attractive young models to highlight plant photos on the cover of *Four Seasons* (Figure 3), and in his "research" papers (e.g., Roof 1972).

Using the *Four Seasons*, he published extensively on his philosophy of *Arctostaphylos* taxonomy. He had rather unconventional ways of thinking about *Arctostaphylos* relationships and later designed *Arctostaphylos* alliances that have not stood the test of time. These were often based on a combination of

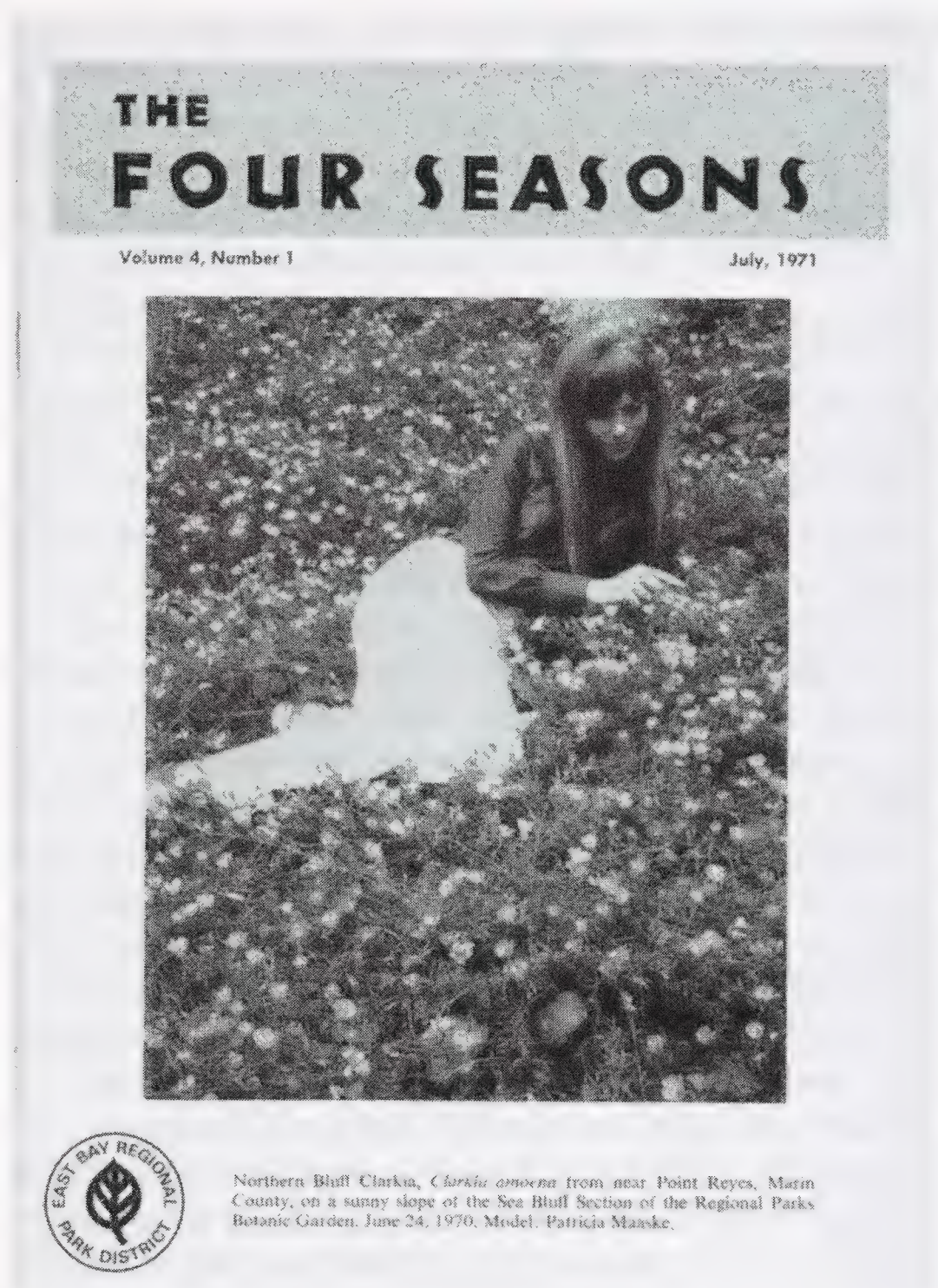


FIG. 3. An example of James Roof's *Four Seasons* (with permission of the East Bay Parks).

morphological and cytological traits, and when he found taxa that did not conform, he would invent colorful terms to describe them (Edwards 1999).

His most extensive writings were on *Arctostaphylos* collections from Pinnacles National Monument and the surrounding landscape of central coastal California. This region is dominated by *A. glauca* and *A. pungens* and Roof described an interesting array of variants, most of which he ascribed to variants of *A. pungens* (Roof 1978). His approach to alliances was to lump together many well-defined taxa such as *A. pungens*, *A. manzanita*, *A. parryana* and numerous other lesser taxa. While we recognize there are some as yet undefined *Arctostaphylos* problems in this central coast region, including potential variation in ploidy level that have not yet been totally resolved, most subsequent botanists have not agreed with his approach. Despite these philosophical perambulations, Roof did describe seven new species of *Arctostaphylos* including three (*A. montaraensis*, *A. cruzensis*, and *A. pacifica*) that are still recognized today (Parker et al. 2012).

Certainly one of Roof's enduring contributions to *Arctostaphylos* was his horticultural and conservation work on *A. densiflora* Baker, a narrow endemic in Sonoma County. Ironically, despite being reduced to just a single small population in the wild, it is without doubt the most widely planted manzanita in the state, thriving in a wide range of conditions. A

collecting party headed by Howard McMinn in 1941 selected an individual they considered best represented the species (Roof 1972) and this is the type from which contemporary cultivars known as “Howard McMinn” were propagated. Roof recalled that for many years this horticultural selection was erroneously referred to as the type specimen of *A. densiflora* but later expressed his “apologies for errors offered and suffered” (Roof 1972, p. 6). Roof was an avid protector of the natural population and not tolerant of offenders, e.g., visiting the roadside population soon after road crews had sprayed engine oil on some of the remaining *A. densiflora*, something he termed “official vandalism,” exclaimed “No savages would have been so heedless of beauty: it was an act of morons” (Roof 1972, p. 6). Roof did much to try to protect this species in the wild although repeated collections from this population by him, Wieslander, Knight and others by transplanting seedlings to their private and public gardens were perhaps misguided since at the time the total known population numbered 41.

James Roof was editor of the Regional Parks botanical journal *Four Seasons* until he had some conflict with the board and created another, very short lived journal, *Changing Seasons*. He bitterly described this as “a necessary replacement for the FOUR SEASONS, the internationally recognized research journal of the now defunct [sic] Regional Parks Botanic Garden” (Roof 1979). Conflicts over the garden earlier in the 1960s, in fact, were critical events leading to the origin of the California Native Plant Society (Stebbins 1990).

Walter Knight (1914–2002) was a great field botanist with a keen eye for manzanitas. He honed his botanical expertise generating plant lists for environmental impact reports dealing with areas planned for development. He also worked for a time at the East Bay Regional Park’s garden. He published a dozen papers; all in *Four Seasons*, on new and interesting *Arctostaphylos* discoveries, and during the years 1966–1985 co-authored a book on the flora of San Bruno Mountain (San Mateo Co) and one on the flora of Sonoma County. He is the author of *A. truei* (Knight 1969), which later was recognized as a subspecies of *A. mewukka* and he was a key contributor to two important species, *A. klamathensis* and *A. malloryi*. Knight was an enthusiastic field botanist and enjoyed going on *Arctostaphylos* forays with others such as James Roof, Steve Edwards, Roman Gankin, and Phil Wells, to name just a few. He was particularly helpful to Philip Wells from 1984 to 1989 while Wells prepared for his 1993 treatment in *The Jepson Manual* (1st edition) (Hickman 1993). Several new taxa were discovered during these trips.

Roman Gankin (1938–) also made important contributions through his dissertation that was a classic on the autecology of the very rare and restricted endemic, *Arctostaphylos myrtifolia* Parry (Gankin and Major 1964). Gankin has always been a

great *Arctostaphylos* explorer and described one of the very unique restricted manzanitas discovered in recent years, *A. refugioensis* Gankin, apparently “scooping” Phil Wells who recognized the unusual characters of this species on herbarium specimens (Wells 2000) but did not make field collections necessary for description. Many of the taxa discovered by this new wave of manzanita taxonomists subsequently made it into the *Supplement to A California Flora* by Munz and Keck (1968). While the academic community was largely convinced that *Arctostaphylos* was “done” after Munz and Keck (1959) (G.L. Stebbins, personal communication), the actions of these field scientists and inclusion in the *Supplement* suggested the contrary. Thus, the “table was set” for the new *Arctostaphylos* “expert” to emerge, Phillip Wells.

PHILIP WELLS ERA

Philip V. Wells (1928–2004) awakened the *Arctostaphylos* community by his appearance with an extensive assessment of chromosome numbers in 1968 that greatly extended our knowledge of manzanita systematics (Wells 1968). Wells was not a native Californian, and he completed his Ph.D. under the great ecologist Dwight Billings at Duke University, sharing an office with another noteworthy California ecologist, Harold A. Mooney. His dissertation was an autecological study of desert tobacco species, and this work seems to have garnered little attention. However, his time in the desert paid dividends because he discovered that ancient desert packrat middens harbored amazing collections of late Pleistocene and early Holocene plant matter that provided an unparalleled opportunity for analyzing temporal shifts in vegetation during past climatic episodes (Wells and Jorgensen 1964). His ability to detect interesting and important ecological patterns extended to studies on the link between Great Plains conifers and fire regimes (Wells 1983), substrate and disturbance impacts on vegetation distribution in central California (Wells 1962), and climatic shifts in chaparral and desert vegetation in central Baja California (Wells 2000). These insights also, of course, contributed to his interest in *Arctostaphylos* (Figure 4), one of the more biogeographically fascinating genera in California.

Wells (1990) credits his interest in *Arctostaphylos* to Cornelius H. Muller. One of his first academic positions following graduate school was a yearlong replacement for Muller at UCSB. In discussions with Muller, he was encouraged to find a genus for taxonomic work that would be a sidelight to his ecological research, an attitude almost certainly offensive to taxonomists who devote their careers to taxonomy (Wells 2000). With Muller this approach was certainly fulfilling in that he continued throughout his career to make occasional contributions to *Quercus* taxonomy, despite his focus on the ecology of allelopathy. With Wells it also turned out

to be a good strategy and as he described it "Although I studied the manzanitas over a period of 32 yr, I really did most of it in concentrated bursts totaling maybe only about three years of full-time activity (maybe enough for 1 Ph.D.)" (Wells pers. comm. to JK).

His contributions to *Arctostaphylos* nomenclature were second only to Alice Eastwood. Wells described 11 new species of which nine are still recognized and two exist as subspecies (100% retention). Wells was also important for three major revisions in the genus in 1968, 1987, and 1988. In 1968, he made 27 new taxonomic rearrangements including adding two new species descriptions (Wells 1968). These were largely driven by his work on *Arctostaphylos* chromosome insights and unfortunately did not make it into the 1968 *Supplement* (Munz and Keck 1968). In 1987, he made six rearrangements including the addition of four trinomials featuring the rank of forma (Wells 1987). Then, in 1988 prior to submission of his taxonomic treatment for *The Jepson Manual* (Hickman 1993), he added 32 new revised taxonomic arrangements (Wells 1988). Species descriptions by Eastwood and Wells combined make up more than one-third (37%) of the currently recognized species (63) in *Arctostaphylos*. Interestingly, neither Eastwood nor Wells included other colleagues as co-authors of their species treatments although both undoubtedly had assistance in their field surveys.

One of the important impacts of Wells' taxonomic work was his decision early on to submerge the rank of variety and to describe all infraspecific taxa as subspecies (Wells 1968). The primary motivation for this was to recognize variation that was geographically circumscribed. However, it is well known that some species, particularly crown-sprouting species such as *A. tomentosa* and *A. glandulosa*, have morphological variants that persist through repeated fire cycles (Keeley et al 2007); to recognize these, and other widespread variants without a clear geographic range, Wells proposed the term forma (Wells 1988).

Wells (1968) presented chromosome counts for over 60 *Arctostaphylos* taxa and used this information to formulate hypotheses on species origins. One notable example was the origin for the tetraploid *A. mewukka* Merriam. Wells hypothesized that this mid-elevation species originated by amphidiploidy from a cross between the higher elevation *A. patula* Greene and lower elevation *A. viscida* Parry. Wells' hypothesis was supported by cytological and genetic work by Kristina Schierenbeck (1956–) (Schierenbeck et al. 1992).

Although Schierenbeck did not pursue a career in *Arctostaphylos* taxonomy she did demonstrate some valuable lessons about the use of manzanitas in forensic science (Schierenbeck 2003). Butte County Sheriff deputies consulted with Dr. Schierenbeck to determine if plant samples found in the back of a pickup truck could assist in tracking down a young girl. A leaf of *A. patula*, in combination with other associated plant species pointed her to a site where

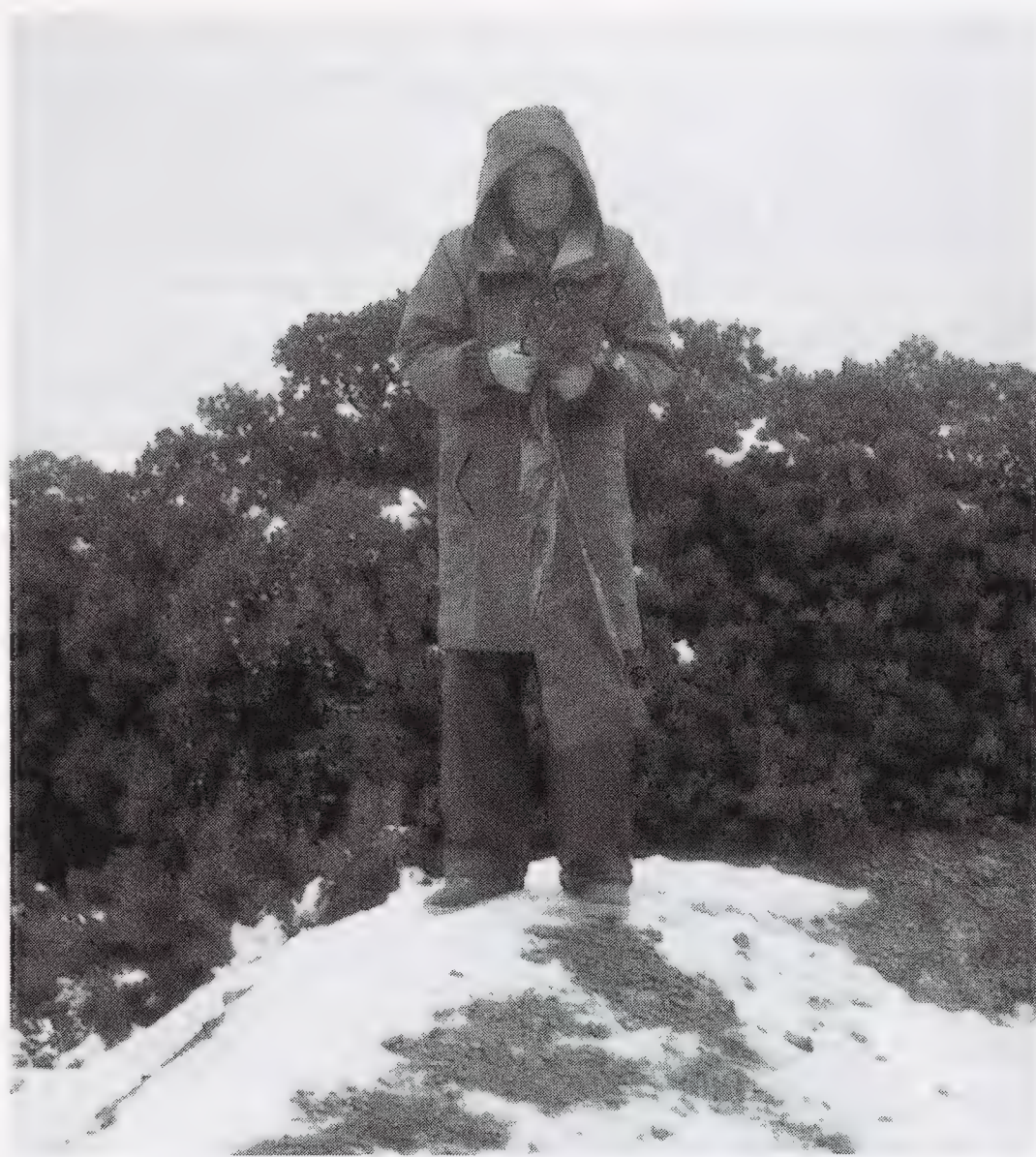


FIG. 4. Philip V. Wells at Mill Creek Summit, Los Angeles County with *A. glandulosa* subsp. *gabrielensis* (P.V. Wells) J. E. Keeley, M.C. Vasey & V.T. Parker, 10 March 1986 (photo by Jon Keeley).

she suspected they came from, and quite unfortunately she led them to the discovery of the young girl's body.

One of the most important contributions by Wells was a paper that discussed obligate-seeding and crown-sprouting species in chaparral (Wells 1969). This work relied heavily on Jepson (1916), whose brilliant insights into the true fire-type shrubs, which were the obligate seeding (Wells' term obligately-seeding) species. However, Wells was able to extend this beyond *Arctostaphylos* to include *Ceanothus* and exclude other genera in chaparral, as well as apply an evolutionary model to explain the advantages of obligate seeding.

Wells did not collaborate with other *Arctostaphylos* aficionados. He did correspond with others (e.g., Walter Knight and Jon Keeley), and used them to guide him on field trips, and discovery of new taxa, but did not include them in his publications. Indeed, type specimens were collected in collaboration with these *Arctostaphylos* experts, but colleagues were never included in his papers or as authorities for his new species. This was perhaps a shortcoming in his work since for the last few decades of his life he had relatively limited access to field work in California and might have benefited from collaboration with active *Arctostaphylos* field botanists.

ABOUT THE AUTHORS

Jon E. Keeley (1949–; first author) grew up in rural San Diego County and spent much of his early childhood exploring the natural history of chaparral

and sage scrub ecosystems around the community of Bonita. He started systematically collecting plants as an undergraduate and pursued a master's degree on postfire chaparral recovery following the massive 1970 Laguna Fire. Philip Wells' paper on the evolution of *Arctostaphylos* and *Ceanothus* life history strategies (Wells 1969) was largely responsible for selecting this thesis topic, and it played a key role in his future career direction (Keeley 2014).

Keeley's keen interest in *Arctostaphylos* evolution was further stimulated by an early paper on hybridization in *Arctostaphylos* written by the renowned Russian emigrant Theodosius Dobzhansky (1953), perhaps most widely known for his maxim "Nothing in biology makes sense except in the light of evolution." Dobzhansky (1953) and UCLA botanist Carl Epling (1947) used a very subjective approach to classifying individuals as hybrids or F1 backcrosses and concluded that *A. patula* and *A. mariposa* (now *A. viscida* subsp. *mariposa*) from near Yosemite National Park were a textbook demonstration of introgression. Although convincing because of their reputation, a clearer demonstration of hybridization was Leslie Gottlieb's (1968, see also Schmid et al. 1968) quantitative analysis of *Arctostaphylos* hybridization. Gottlieb went on to have a very successful career as a plant geneticist, however to some of us he is best remembered for his insightful essay contrasting Herman Melville's and Charles Darwin's reaction to the Galapagos Islands landscape (Gottlieb 1975). Keeley (1976) applied a similar approach to a mixed population of *A. glauca* and *A. pungens* in San Diego County and presented evidence not of just plants with intermediate characteristics of both putative parental species, but also individuals that recombined combinations of traits from both species, highly suggestive of hybridization and introgression.

Keeley's interest in *Arctostaphylos* further developed through his explorations and discovery of new taxa in Southern California (Keeley et al. 1997a) and Baja California (Keeley et al. 1997b, Keeley et al. 2007). One early specimen from northern San Diego County was sent to Wells and he disagreed with Keeley's assessment and claimed it was just a range extension of *A. peninsularis*, a Baja species, one which Wells had recently named (Keeley 1974). Eventually, Keeley realized it actually was a new species and named it *A. rainbowensis* (Keeley and Massihi 1994). Wells, however, continued to insist he was correct, and in his 2000 monograph, submerged *A. rainbowensis* as a subspecies of *A. peninsularis* (*A. peninsularis* subsp. *keeleyi*). Having known Phil Wells personally it is hard to not believe that this is similar in intent to the story of *A. jepsonii* Eastwood, recounted in the opening paragraphs.

In 1985 Virgil Thomas Parker attended his first workshop on *Arctostaphylos* at UCSB because of an interest in resprouting and seeding life histories in *Arctostaphylos*. The guest speakers at the workshop were Philip Wells (University of Kansas) and Jon

Keeley (Occidental College in Los Angeles), and each were given 50 min to speak and were told they had to adhere closely because the room had to be vacated immediately after the two talks. In a style later recognized as typical Wells, at 50 min Phil wasn't close to finishing and turned to Keeley rhetorically saying "you won't mind me taking some of your time" and without waiting for an answer continued for another half hour. Perhaps one of the better parts of this meeting, though, was the after lunch lab display of a rich diversity of live *Arctostaphylos* collected by Wayne Ferren and other UCSB botanists. It was here that Keeley and Parker had a chance to meet and they have continued to correspond on similar interests in *Arctostaphylos* taxonomy and ecology over the following years.

Tom Parker and Michael C. Vasey (1947–) got serious about *Arctostaphylos* taxonomy following a 1992 discussion between Vasey and an editor for the *Flora of North America* (FNA) project, in which some concerns were raised about turning the FNA *Arctostaphylos* treatment over to Philip Wells, the natural heir since he had recently completed the *Arctostaphylos* treatment for the 1993 *Jepson Manual* (Hickman 1993). Apparently, word had reached them of difficulties in dealing with Wells and particularly his insistence upon using a trinomial nomenclatural system (including the rank of *forma*) for several important groups (Wells 1988), although *forma* was never used in *The Jepson Manual*. As a consequence, the editors were willing to consider alternatives. This FNA representative recognized Vasey was very knowledgeable on the genus and challenged him to do the treatment. Vasey agreed as long as he could collaborate with Parker, and, in order to do more than just "rearrange the deck chairs," FNA needed to help them investigate molecular data with funding, which subsequently did come through. During these early studies in 1990, there was a discussion between Parker and Keeley about ways to collaborate on *Arctostaphylos* problems. In 1994, Parker suggested that he and Keeley collaborate on a National Science Foundation research proposal to further the understanding of *Arctostaphylos* evolution through molecular methodologies. However, this proposal was not funded and this rejection instilled in Parker the need for collecting "proof of concept" data that would ultimately allow a fuller understanding of the phylogeny of this group.

Ultimately, the first study using nuclear ribosomal DNA in *Arctostaphylos* (Markos et al. 1998) showed promise for altering our view of *Arctostaphylos* evolution as it raised the likelihood that Wells' perspective on the phylogeny of the group was in need of reexamination. Throughout Wells' career, he expressed strong opinions on the appropriate subgeneric classification system and species affinities. His morphologically based cladistic analysis employed 70 traits that he contended pointed clearly to two subgenera, each with three sections, and these conclusions were presented in Wells (2000), but to our knowledge the cladistic analysis was never

published. The subsequent molecular work (Markos et al. 1998, Hileman et al. 2001, Boykin et al. 2005, Wahlert et al. 2009) provided evidence to revised Well's *Arctostaphylos* phylogenetic concepts.

This molecular rDNA work also cast light on evolution within the subgenus *Arbutioideae*. Particularly surprising was the discovery that although *Arbutus* is a close relative to *Arctostaphylos*, manzanitas are more closely related to Mediterranean *Arbutus* species than to North American *Arbutus* (Hileman et al. 2001). This unexpected finding, however, is consistent with phenological patterns of flowering. *Arctostaphylos* flower from nascent inflorescences on old wood from the previous year, and this is also the pattern for Mediterranean *Arbutus*, but not with North American *Arbutus*, which do not produce persistent nascent inflorescences and flower from new growth (Keeley 1997).

Another direction Parker and Vasey took was holding 2-day *Arctostaphylos* workshops for manzanita aficionados, most often hosted by the Jepson Herbarium and held at Hastings Natural History Reservation in the Carmel Valley. These outings not only made this difficult genus more accessible to non-specialists but the specialists seemed to learn more and more about manzanita mysteries with each workshop. The most significant contribution was the development of a workable key to the genus, polished by participants over the years in these workshops.

By the early 2000's the FNA project was still in the works and now the invitation for *The Jepson Manual* revision came through. In light of California's large latitudinal range there are substantial differences between the north and the south. Parker and Vasey, realizing they needed help on the southern California manzanitas, contacted Keeley about providing input and collaboration, which seemed like the best solution (Figure 5). One of the first collaborative projects was an attempt to sort out the complex of *A. glandulosa* interspecific variation (Keeley et al. 2007). Using a collection of 1400 sheets of this species, based on a decade of collecting from Oregon to Baja California, they were able to make quantitative trait comparisons that sorted out the taxa and interpreted the results as comprising two lineages, a glandular and non-glandular line, each of which have given rise to various subspecies. The next collaborative effort was a paper presenting a number of changes in taxonomy within the genus (Parker et al. 2007), necessary before incorporating these changes into the final treatment. What was most interesting about these two papers was the review process. In the case of the former, which used large datasets and statistical analyses, the paper invoked substantial review critiques that were rather difficult to address. The latter paper involved just expert opinion and received almost no critiques and was published more or less as is. Both *Arctostaphylos* treatments were eventually published (Parker et al. 2009, 2012). This collaboration continues with the most recent project one that has addressed Jepson's

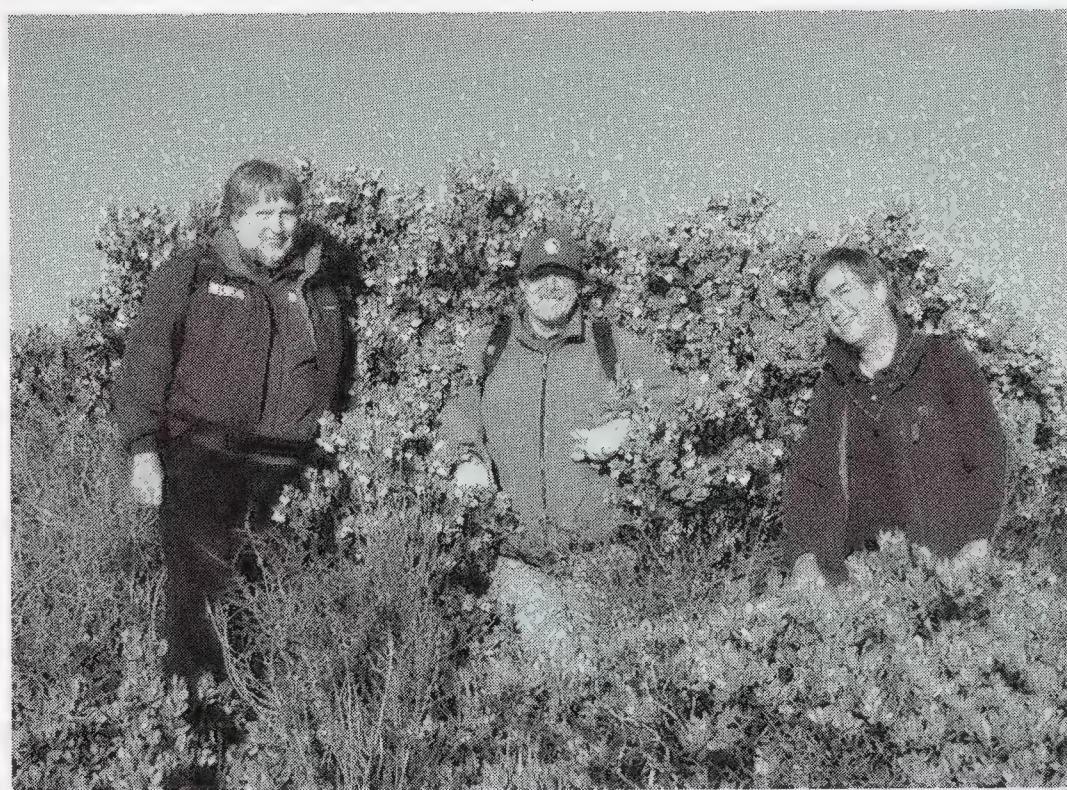


FIG. 5. Tom Parker, Mike Vasey, and Jon Keeley at type locality (Gabilan Range, San Benito Co.) for *A. gabilanensis* circa 2005 (photo by Jon Keeley).

(1916) early interest in resprouting and seeding 100 yr later (Keeley et al. 2016).

CONCLUSIONS

Over the past three centuries, nearly fifty authors have described over three hundred *Arctostaphylos* taxa. Our principal objective has been to introduce others to the small group of those individuals who have been most involved in the naming of species in this charismatic genus. Undoubtedly, other complicated genera have similar humans that were part of the history of systematic research and we encourage researchers to also investigate the history of their preferred organisms, as it will be fruitful from a research perspective, as well as insightful about the course of human endeavors within challenging groups. Early on, we thought that this might be an exposition on the advantages of collaboration versus competition in science, but clearly examples of both arise throughout the history of this group. Indeed, we are convinced that one thing held in common with most if not all who pursue manzanita studies is the pleasure of joining others in field explorations in such a rich and diverse environment as the California Floristic Province. We feel that considerable progress has been made on understanding the evolution and diversity of this impressive genus, and yet we suspect that the diversity of personalities working in the group has probably been as important an element in that progress as other factors.

We end with a few quotes that summarize to us some of the lessons gleaned from this the history of *Arctostaphylos* taxonomy. "The basic trouble, you see, is that people think that 'right' and 'wrong' are absolute; that everything that isn't perfectly and completely right is totally and equally wrong. However, I don't think that's so. It seems to me that right and wrong are fuzzy concepts, . . ." (Asimov 1989, p. 35). "An expert is a man who has made all

the mistakes which can be made, in a narrow field.” (Niels Bohr as quoted by Edward Teller in Coughlan 1954, p. 62). “If we knew what we were doing, it wouldn’t be called ‘research’, would it?” (Albert Einstein as quoted by Hawken et al. 1999, p. 272). It seems these characters and their philosophies perfectly capture the on-going reality of work in a genus as challenging and fascinating as *Arctostaphylos*. We have no doubt that other authorities will emerge, new insights will be gleaned, and different arrangements of relationships will be fashioned based upon other perspectives. In this era of shifting taxonomic identities, it is well to keep in mind that species are always hypotheses of phylogenetic relationships based upon the best information and interpretation of data available at a given time. Taxonomic characters shape these interpretations but it is the interplay of human characters, with all their various foibles and differing strengths, which ultimately shape taxonomic treatments! Most importantly we need to keep in mind that the true ‘peer-review’ process is something that unfolds over years or decades following publication of a scientific paper.

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CBS PRESIDENT'S REPORT FOR VOLUME 64

Dear Colleagues,

This is my first report as President of the Society, and I'm delighted to represent the Society as the Council as I develop new opportunities for our members. We are now planning how to implement several new initiatives to build membership in the Society; to enhance botanical careers; to create new avenues for collaborative research and education; and, of course, to reduce plant blindness near and far. *Madroño* is under new leadership as well; Dr. Justen Whittall (Santa Clara University) is the journal's incoming Editor, and I'd like to express my deep gratitude to Dr. Matt Ritter (Cal Poly, San Luis Obispo), *Madroño*'s Editor from 2012–2016, who is now introducing Justen to the tasks at hand. Continue to look for the arrival of your quarterly issues!

The 100 of us who attended the 2017 Graduate Symposium at the Santa Barbara Botanic Garden in April couldn't help being impressed by the enthusiasm, knowledge, creativity, and diversity of the participating graduate students, postdocs, and faculty. The symposium included 30 presentations, 16 posters, and 60 people at the banquet: a very full day in the SBBG's brand new and stunning Pritzlaff Conservation Center, and among the Garden's botanically rich trails.

The spring also brought the Council's decisions regarding the 2017 Paul Silva Student Research Grant awards. The competition was so stiff that three awards were granted, including one to a four-student team from Cal Poly (read more about them in the upcoming issue of the Society's newsletter, *Nemophila*, and at calbotsoc.org/grants/). In light of all of this happy activity, it was easy to feel that the future of botanical research in California is in excellent hands, but with the unpredictable effects of climate change looming, and with insufficient funding for basic and applied research — not to mention for the kind of far-ranging field courses that drew many of us into Botany — we must be mindful to keep this future secure.

We know two things for certain: wild plants and their habitats need the attention of all plant scientists, and, in order to create the next generation of talented and sharp-eyed botanists, we must encourage them (and perhaps ourselves, too...) to look up from their hand-held devices and introduce them to the pleasures and adventures intrinsic to discovering and observing wild plants — and their pollinators! — in their natural habitats.

As the only statewide organization dedicated to developing a pipeline for the training of professional

plant scientists and academicians in California, the Society is uniquely poised to facilitate the growth, skills, and visibility of botanical experts whose work contributes to the persistence and diversity of our state's flora. Towards this goal, the Council has assembled a set of initiatives for the upcoming year, including the following priorities:

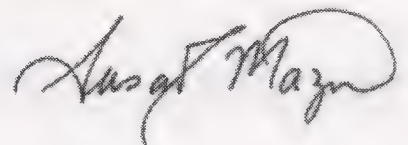
Botany Ambassador Program: This program aims to inspire future botanists by creating bridges between graduate students, informal science education programs, and local classrooms. Participants will develop and exchange teaching materials (to be made available on our website), and visit local schools, botanic gardens, and outdoor education venues to introduce K–12 students to botany and to the wide range of botanical careers that they may pursue. Participants will also have the opportunity to “translate” recent *Madroño* articles into short summaries for general audiences, to be posted on the Society's website. In short, we aim to ignite young students' interest and awareness of botany as a career, and to facilitate the professional training of current graduate students. Those interested in participating or supporting this program should contact our Membership Chair, Rachael L. Olliff Yang (membership@calbotsoc.org).

Membership growth and retention: California has an abundance of riches with respect to botanical societies (can you name at least three others?), so the California Botanical Society must rise to the challenge of continuing to build our membership if we are to grow and to thrive. The Council is considering a number of ways to entice new members to join, including: facilitating professional networking through our website; creating a portal through which students and investigators may seek new collaborators; reaching out to professional botanists at universities and colleges across California and other western states; increasing the availability of funds for undergraduate and graduate student research; and strengthening our relationships with western botanical gardens.

Professional development: The Council wishes to offer a new training opportunity for graduate students and postdoctoral researchers by inviting them to review manuscripts submitted to *Madroño* and to learn from the comments provided by other reviewers as well as from the Editor's decision. If you would like to participate in this opportunity, or if you are a faculty member with lab members who you would like to recommend for this activity, please contact incoming *Madroño* editor, Dr. Justen Whittall (madronoeditor@gmail.com).

The Council is brainstorming about other ways to increase the value of the Society (and our website) to our members, and, with a bit of luck, you'll hear a lot more about these plans at the 2018 California Botanical Society Banquet to be held at UC Davis – but only if you come (better yet, bring a new member, too)! To that hopeful end, please keep an eye out for upcoming announcements.

With best wishes for a cool and crisp autumn,



Susan J. Mazer, August 2017
Professor of Ecology and Evolution
University of California Santa Barbara
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EDITOR'S REPORT FOR VOLUME 64

We are pleased to report the publication of volume 64 of *Madroño* by the California Botanical Society (CBS) in 2017.

Madroño editorship is transitioning from Matt Ritter (Cal Poly San Luis Obispo) to Justen Whittall (Santa Clara University). We have enjoyed co-editing the last several issues in this volume during the transition. Matt has been the editor for the past five years bringing the journal back on schedule while maintaining a high quality publication. His drive to keep *Madroño* at the forefront of natural history publications while arriving in your mailbox on time is inspiring.

Madroño remains on schedule with an average time between initial submission and publication of about 8 mo. We believe that *Madroño* is still the best outlet for western botanists to publish their discoveries in a timely fashion, while reaching an interested and relevant audience. We hope to grow the

Madroño readership in the upcoming year with increased online accessibility.

The efforts of numerous individuals are critical to the continued quality of the journal. Thank you as well to the Noteworthy Collections editor, David Keil. Steve Timbrook has long provided the Volume Index and Table of Contents for the journal and we thank him for his efforts. We thank Lynn Yamashita for providing administrative support at critical times. We are also grateful to our reviewers who regularly provide insightful reviews bettering our publication.

Editing *Madroño* is a joy. Being on the forefront of western botany and working with so many wonderful botanists as authors and reviewers is a pleasure. Botany is alive and well in Western North America! The evidence is in *Madroño*. Please continue to submit your work to the journal and have a great year.

Matt Ritter & Justen Whittall

August 2017

REVIEWERS OF MADROÑO MANUSCRIPTS 2017

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SUBSCRIPTIONS—MEMBERSHIP

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MADROÑO

A WEST AMERICAN JOURNAL OF BOTANY

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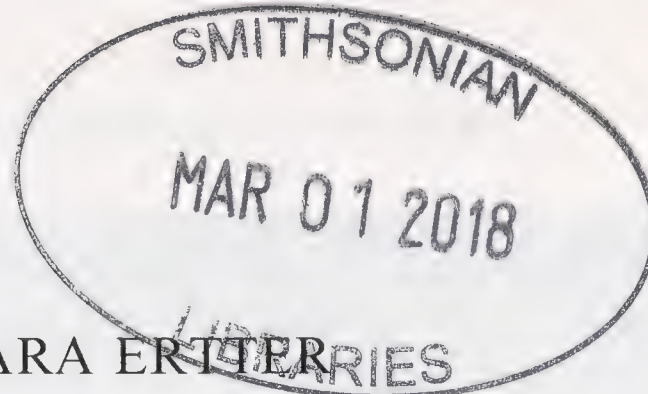
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DEDICATION TO BARBARA ERTTER

Dr. Barbara Ertter, Curator of Western North American Flora at the University and Jepson Herbaria at the University of California (UC), Berkeley, is more than worthy of the dedication of this issue of *Madroño*. She is a consummate botanist who is highly intelligent and productive. She has moved two major herbarium collections, published many botanical revisions including descriptions of numerous new species and infraspecific taxa, and has species named in her honor for their discovery. Through workshops and lectures, Barbara has shared her extensive knowledge of Rosaceous genera, aquatic plants, weeds, the history of botany, and other topics. She has encouraged others to study and publish newly discovered taxa, has assisted many colleagues with their specimen needs for the two editions of *The Jepson Manual*, and through publication of her *East Bay Checklist* and the revised *Bowerman Flora of Mount Diablo*, she has contributed much to the understanding of California floristics.

Barbara grew up in Boise, Idaho, and was always interested in nature. By the time she was in college, Barbara was actively and academically exploring her botanical neighborhood under the mentorship of Pat Packard, herself an excellent botanist, at the College of Idaho. Upon her graduation in 1975, her summa cum laude performance as an undergraduate was rewarded by acceptance into the systematic botany program at the College of Maryland where she studied under Jim Reveal and produced a “Revision of *Oxytheca*, an annual derivative of *Eriogonum* centered in southern California” which included two new taxa: *Oxytheca parishii* Parry var. *goodmaniana* Ertter and *O. parishii* Parry var. *cienegeensis* Ertter. She went on to study at the City University of New York in a joint program with New York Botanical Garden under the great Art Cronquist, and acquired her PhD in 1983. She worked on a group decidedly lacking in glamor: the species of *Juncus* (Juncaceae) that one normally doesn’t see, but rather treads upon. This work resulted in a “Revision of the *Juncus triformis* complex, a group of dwarf rushes centered in California” which included three new taxa: *Juncus luciensis* Ertter, *J. tiehmii* Ertter, and *J. leiospermus* F.J. Herm. var. *ahartii* Ertter.

Barbara’s academic training allowed her to move one step back west: from 1982 to 1985 she worked as Collections Manager at the herbarium at the University of Texas at Austin (UT), under the direction of Billie Turner. While there, as well as learning the local flora and executing her curatorial duties, Barbara broadened her botanical interests to the challenging Rosaceous genera *Ivesia* and *Horkelia*, ultimately resulting in her 1989 publication “Revisionary studies in *Ivesia* (Rosaceae: Potentilleae).”

One of Barbara’s jobs at UT was to move the herbarium into new quarters: no small job, as the collection consists of approximately 800,000 specimens. Because of this experience, she was a perfect candidate for her next position at the University of California, Berkeley as Collections Manager

for the University and Jepson herbaria (UC/JEPS). Shortly after moving to Berkeley in 1985, Barbara’s challenge was to plan and execute a move of this large herbarium (approximately 1,800,000 specimens) into temporary headquarters, to work with others to design a new herbarium, and then to plan and execute the move of the herbarium back into the newly renovated Valley Life Sciences Building. Barbara spearheaded this move all while keeping up with day-to-day use of the facility and loan requests during a busy time at UC/JEPS, as 1985–1993 were the years when scores of botanists were working on the 1993 edition of *The Jepson Manual*.

During these years, UC/JEPS was a botanically exciting place: with botanical greats such as Bob Ornduff, Larry Heckard, Lincoln Constance, Lauramay Dempster, and Annetta Carter to name a few, and Barbara was close to and very supportive of Lincoln Constance and Annetta Carter, especially in their later years. She collaborated with many botanists who visited the herbarium or were research affiliates, and her support and collaboration resulted in documented range extensions and new species descriptions from localities throughout California, including coauthoring the description of a new species in the genus *Neviusia* (Rosaceae). The new species, *N. cliftonii*, was discovered in Shasta County from a genus previously only known from the southeastern United States.

Barbara was also a botanical diplomat through her work with Dr. Fosiee Tabhaz, a colleague in the herbarium who was from Iran but was forced to move to the US because of the revolution. Barbara and Fosiee put together trips to Iran, where they botanized, interacted with academics, and gave presentations (read more about the Joint Program in Botany between Iranian Institutions and University Herbarium at: http://ucjeps.berkeley.edu/main/research/iran/IRAN_GLOBE.final.pdf).



FIG. 1. Barbara Ertter, 2002, at the UC/JEPS signing a copy of *The Flowering Plants and Ferns of Mount Diablo*. Photograph by Staci Marcos.



FIG. 2. Barbara Ertter, 2012, on the flanks of Slate Peak, North Cascades, Washington. Photograph by L. A. Vorobik.



FIG. 3. Barbara Ertter, 2012, vicinity of Slate Peak, North Cascades, Washington: a botanist at work. Photograph by L. A. Vorobik.

While at Berkeley, Barbara contributed greatly to the conservation and understanding of East Bay plants. She worked with the East Bay Chapter of the California Native Plant Society and Save Albany Hill, produced a detailed checklist of all vascular plants of the East Bay, and worked for many years with Mary Bowerman on a complete overhaul and republication of Bowerman's flora of Mount Diablo, published in 2002.

More recently Barbara has continued her work in Rosaceae, studying the large and difficult genus *Potentilla*. The results of years of research are her treatment of this and related genera in *Flora North America: Rosaceae*.

Among her many qualities, Barbara is a great hiker, loves the outdoors, and is a voracious reader, thinker, and writer, whose every thought is philosophical on some level. She has a quirky sense of humor and an annoyingly excellent memory. Barbara's appreciation and understanding of botany is a magnet that has and will draw those of a like mind to her, for a shared session over a mysterious specimen, a challenging conversation about how best to understand relationships among species, or for an amazing hike in a wild area sleuthing out the location of some *Potentilla* or other species that might unlock the secrets to the current taxonomic question on her mind.

—LINDA ANN VOROBİK, The University and Jepson Herbaria, 1001 Valley Life Sciences Bldg. 2465, University of California, Berkeley, CA 94720-2465.

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NOTEWORTHY COLLECTION

ARIZONA

LOPHOCEREUS SCHOTTII (Engelm.) Britton & Rose (CACTACEAE).—Yuma Co., Barry M. Goldwater Range-West (BMGR-West), Raven Butte 7.5' USGS quad, 32.4741°, –114.0738° (WGS84), 210 m (690 ft) elevation, approximately 57 km ESE of Yuma, 5 October 2016, R. P. O'Donnell with V. Luois, R. L. Harrow, and S. L. Arnett-Romero (Desert Botanical Garden DES00083297). One individual approximately 5.6 m tall \times 4.4 m wide, on sandy alluvium between the braids of Coyote Wash and approximately 90 m E of the main channel, in the Lechuguilla Valley, between the Copper and Gila Mountains, in lower Sonoran Desert habitat dominated by *Larrea tridentata* (DC.) Coville. BMGR-West is managed by the Marine Corps Air Station Yuma in Yuma, AZ.

Previous knowledge. This columnar, multiple-stemmed cactus is predominately found in Baja California, Sinaloa, and Sonora, Mexico. In the United States, this species occurs almost exclusively in Organ Pipe National Monument of southern Arizona (Parker 1989). Several individuals have also been reported approximately 5 km west of the western boundary of Organ Pipe National Monument in Bell Pass, Agua Dulce Mountains, Cabeza Prieta National Wildlife Refuge (Felger et al. 2014). Recently, two individuals were discovered near Corn Springs in the Little Chuckwalla Mountains, southeast California, the first record of this species in the state (Cloud-Hughes and Baker 2014). We describe the discovery of an individual *Lophocereus schottii* located a significant distance from previous records, in the Barry M. Goldwater Range-West. While the Barry M. Goldwater Range-West has been extensively surveyed for plants, including 656 sample sites and 5200 km of visual surveys (Malusa and Sundt 2015), this species had not been previously reported in this area, or anywhere in Arizona away from Cabeza Prieta National Wildlife Refuge or Organ Pipe National Monument.

Significance. This is the northernmost and westernmost occurrence of *Lophocereus schottii* in Arizona, representing a range extension within the state of approximately 100 km WNW from both Organ Pipe National Monument and from Bell Pass in the Cabeza Prieta National Wildlife Refuge. This record helps fill the 260 km gap between records from southern California and southern Arizona, being about 170 km from the former and 100 km from the latter.

While Cloud-Hughes and Baker (2014) suggested that the California individuals might have been

planted as ornamentals in the last 50 yr, we think that explanation is unlikely for this individual. There are limitations to estimating the age of cacti due to significant variation in environmental conditions across sites (Drezner and Lazarus 2008), but data from long-term growth measurements of *Lophocereus schottii* on Organ Pipe National Monument (Parker 1988) suggest that this cactus is probably much more than 43 yr old, the approximate maximum age modeled by Parker (1988). Indeed, this individual, which is relatively large, presumably occurs in suboptimal conditions as it is at the edge of its range; whereas those individuals studied by Parker (1988) occur at the center of their distribution in Arizona and likely have faster growth rates due to more favorable environmental conditions. *Lophocereus schottii* is long-lived, living to approximately 75 yr (Shreve 1935). Given that this individual was large, old, and in a relatively remote location (17 km from the nearest residence in the town of Wellton and 3.4 km from the nearest dirt road) we consider it unlikely that it was recently introduced by humans.

The present discovery raises the possibility of a previously greater range of *Lophocereus schottii* and that this individual and those from Corn Springs and Bell Pass are relicts of a once more widespread population. Like most Sonoran Desert flora, *Lophocereus schottii* is frost intolerant, and its range has likely experienced southward contractions and northward expansions in response to geologic periods of climatic cooling and warming (Nason et al. 2002). In addition, populations of columnar cacti such as *Lophocereus schottii* are characterized by dramatic natural fluctuations in their numbers (Drezner and Lazarus 2008). Thus, relict individuals and relict populations are expected at the edges of this species' range.

Another possibility is that this individual was introduced to its present location via zoochory. Within the 50 km-long Lechuguilla Valley, Coyote Wash is a major corridor for both xeroriparian plants and wildlife between Sonora, Mexico and the Gila River, Arizona. Since the fleshy fruits of columnar cacti are attractants for animals including birds and mammals (Fleming and Valiente-Banuet 2002; Felger et al. 2014), it is possible that a *Lophocereus schottii* seed was dispersed by a migrating animal. In addition, Native Americans are known to have used and consumed the fruit of this species (Felger and Moser 1974). This cactus might descend from seeds accidentally dispersed by Native Americans, who were present in the area and used Coyote Wash from the late Pleistocene into historic times (Hartmann et al. 2012). Thus, this individual might represent either a relict of a once wider distribution,

or a pioneer (or descendent of a pioneer) brought north decades or centuries ago by a dispersing human or other animal.

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NOTEWORTHY COLLECTION

CALIFORNIA

OXYTROPIS OREOPHILA A. Gray var. *JUNIPERINA* S.L. Welsh (FABACEAE). Mono County, eastern Mono Valley, 8.5 km SE of Cedar Hill, 3 km S of Nevada Hwy 359, on both sides of the California-Nevada state line, 38.14063889 N, 118.77027778 W (WGS84), 2154 m, 13 June 2017, *Ann Howald* 4237 with *Arnold Tiehm* (UCR). About 100 plants present, on the south slope of a rounded ridge of hydrothermally altered gray clay and sand overlain by angular, white gravel (likely siliceous sinter, opaline silica deposited from mineral springs [G. Stock, Yosemite National Park, personal communication]), with *Juniperus osteosperma* (Torr.) Little, *Ericameria parryi* (A. Gray) G.L. Nesom & G.I. Baird var. *nevadensis* (A. Gray) G.L. Nesom & G.I. Baird, *Phlox condensata* (A. Gray) E.E. Nelson, *Oenothera cespitosa* Nutt. subsp. *crinita* (Rydb.) Munz, *Asclepias cryptoceras* S. Watson, *Mentzelia torreyi* A. Gray var. *torreyi*, and *Cryptantha humilis* (A. Gray) Payson. A taprooted, densely matted perennial herb; mats 2 cm tall, up to 1.5 dm wide; banner pale lilac, wings and keel purple.

Previous knowledge. Barneby (1989) describes the distribution as “discontinuously dispersed from se. Nev. (s. Eureka, n. White Pine, e. Lincoln cos.) to sw. Utah (w. Iron and w. Beaver cos.), reappearing on e. foothills of the Utah Plateaus in sw. Sevier, se. Piute, adj. Wayne, and c. Garfield cos.” Welsh (2001) describes the distribution as “Nevada and Utah.”

Significance. This is the first report for California, and a western range extension of about 210 km from the nearest known locality in Nevada, in Little Fish Lake Valley, Nye County. The site in California is the same area and substrate from which *Physaria ludoviciana* (Nutt.) O’Kane & Al-Shehbaz and *Astragalus kentrophyta* A. Gray var. *ungulatus* M.E. Jones previously were reported as new to California (Tiehm 2007). Interestingly, *Physaria* and *Oxytropis* have been found in adjacent Mineral County, Nevada, whereas the *Astragalus* has not (SEINet accesses through <http://intermountainbiota.org/portal/collections/list.php> on 6 July 2017).

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NOTEWORTHY COLLECTIONS

CALIFORNIA

RANUNCULUS CANUS Benth. var. *LUDOVICIANUS* (Greene) L.D.Benson (RANUNCULACEAE). — San Luis Obispo Co., Highway 41 between Shandon and Creston at gate to 3045 Highway 41, north-facing hillside with foothill woodland, *Quercus lobata* Née dominant above road; many annual and perennial herbs beneath and between oaks, scattered on north facing slope in partial shade or in openings among oaks, 35.578125°, –120.447997°, 390 m, 4 April 2017, David Keil 34378 (OBI); Highway 41 between Creston and Shandon at 3220 Highway 41, foothill woodland and old almond orchard, scattered oaks, herbaceous layer with various annual and perennial wildflowers, locally common, 35.579753°, –120.453712°, 400 m, 4 April 2017, David Keil 34380 (OBI); Coast Range, road between Creston and Shandon, c. 9 miles SW of Shandon (Shedd Canyon 7.5' Q, 35°34'37"N [35.576944°], 120°27'49"W [–120.463611°] (±1000 m), 427–457 m, 11 May 1963, F. C. Vasek 630511-02 (UCR4408).

Previous knowledge. Greene (1886) described *Ranunculus ludovicianus* based on collections from “High valleys among the mountains of San Luis Obispo Co., California, and eastward to Tehachapi Pass. Collected by Mrs. Curran, in 1884”. The epithet “ludovicianus” is a derivative of “Ludovicus,” Latin for Louis or Luís and was apparently used by Greene as a reference to San Luis Obispo Co. The plant was soon treated as a variant of more widespread species. Gray (1886), apparently without having seen the specimens described by Greene, proposed a vaguely characterized *Ranunculus californicus* Benth. var. *latilobus* A.Gray and included Greene’s *R. ludovicianus* as a synonym. Greene (1892) published *R. californicus* var. *canescens* Greene and commented that it “belongs to the middle elevations of the Mt. Diablo Range and the valleys among them, from Niles to the hills east of Livermore, thence southward to San Luis Obispo Co. It was part of my *R. Ludovicianus*.” Greene’s (1892) restriction of *R. ludovicianus* to the plants ranging from Kern Co. to San Bernardino and his exclusion of the plants from San Luis Obispo Co. is a clear indication that he had based the name on at least two specimens. Robinson (1895) recognized Gray’s *R. californicus* var. *latilobus* as “a common form, especially southward, from San Francisco Bay to San Diego and San Bernardino,” with *R. ludovicianus* treated as a synonym. He commented about var. *latilobus* that “some forms too nearly approach *R. canus*.” Robinson further noted that “a number of further varieties of the polymorphous *R. californicus* have been characterized by Prof. Greene, Fl. Francis. 299, & Erythea, i. 125; the material at

hand, however, fails to show these forms well-marked among frequent intermediates.”

Davis (1900) proposed *R. californicus* var. *ludovicianus* (Greene) K.C.Davis, a treatment that was followed by Jepson (1922, 1925) and Benson (1936). Benson (1936) effectively lectotypified Greene’s *R. ludovicianus* by citing the type collection as: “High Valleys among the mountains of San Luis Obispo Co., California,” and the collector as “Mrs. Curran (Brandeggee).”

Benson (1941) revised his opinion and treated Greene’s buttercup as *Ranunculus canus* Benth. var. *ludovicianus* (Greene) L.D.Benson. Benson attempted in vain to locate type material of *R. ludovicianus*: “Neither the Brandeggee Collection at the University of California nor the Herbarium Greeneanum contains a specimen collected by Mrs. Curran (Brandeggee) in San Luis Obispo Co., and no specimen of this variety has been collected in San Luis Obispo Co. at any other time. The epithet *ludovicianus* has been consistently applied to the common many-petalled plant growing in the Tehachapi Mountains, and the writer believes that, in the absence of a type, established custom should be law. The 1884 collection from Tehachapi by Mrs. Curran (Brandeggee) is designated as a LECTOTYPE, and, in the event that it is not rediscovered, the 1895 collection by Mrs. Brandeggee (Herbarium of the University of California) should be considered a substitute for it. The 1884 specimen is to be found in neither the University of California Herbarium nor the Herbarium Greeneanum.” Benson (1948) formally designated an 1895 collection by Mrs. Brandeggee from Tehachapi as the neotype for *R. ludovicianus* (UC205003; isoneotype, POM11643), thus fixing the application of the name with the plants of the Tehachapi Range [unless the 1884 San Luis Obispo Co. specimen collected by Curran were to be relocated]. A second isoneotype in the Stanford University Herbarium (DS197721 = CAS-BOT-BC378843) is housed at the California Academy of Sciences (CCH 2017).

Where are the missing type specimens? A search of the Consortium of California Herbaria database (CCH 2017) uncovered no collections whatsoever made by Katharine Curran in 1884 from San Luis Obispo Co. and only a couple from Tehachapi in that year. At the time of Greene’s publication of *R. ludovicianus*, Curran was Curator of Botany at the California Academy of Sciences and editor of the Bulletin of the California Academy of Sciences (Daniel 2008, Carter 2009), and her collections from that time would be expected to be either in the herbarium of the California Academy of Sciences or in her personal herbarium. For a time Greene worked as a fellow curator with Katharine Curran

in the Academy herbarium (Carter 2009, Daniel 2008), and it was likely during this time that he examined her 1884 collections; he published his new buttercup in the Bulletin. In subsequent years Katharine Curran [Brandege after 1889] and E. L. Greene became bitter enemies (Carter 2009, Daniel 2008). Katharine and her husband T. S. Brandege together amassed an herbarium of their own estimated to include about 100,000 specimens (Anonymous 1906), that was donated to the University of California in 1906. Benson (1948) did not locate any type material in the Greene Herbarium (ND-G). Greene also deposited a few specimens in the herbarium of the Catholic University of America (LCU), which was dispersed by sale in the 1980s (Tucker et al. 1989); but none of the herbaria that acquired type or Ranunculaceae specimens from LCU (CM, NA, WIS) houses any of Curran's *Ranunculus* specimens (Carnegie Museum of Natural History 2017; Mid-Atlantic Herbarium Consortium 2017; USNA 2017; M. A. Feist, pers. comm.). The absence of the 1884 collections from San Luis Obispo Co. and Tehachapi from the Brandege and Greene herbaria means that it is probable that the specimens were deposited in the California Academy of Sciences herbarium and were destroyed in the 1906 San Francisco earthquake and fire that consumed most of the collections of the California Academy of Sciences, a conclusion also reached by Twisselmann (1967).

Several early floristic treatments (Greene 1892; Jepson 1922, 1925) recognized *R. californicus* var. *ludovicianus* as a taxon restricted to the Tehachapi Mountains or ranging to the San Bernardino Mountains (and in some reports to San Diego Co.). Either the exclusion of San Luis Obispo Co. from the range of the taxon initiated by Greene (1892) was followed, or in the absence of any additional specimens from the county, the initial report was ignored. Subsequent floras (Abrams 1944; Munz and Keck 1959; Munz 1974; Whittemore 1997, 2012), following the taxonomy of Benson (1941, 1948), treated the plant as *R. canus* var. *ludovicianus*. Hoover (1970) did not mention var. *ludovicianus*, but in his discussion of *R. californicus* he commented that "some plants, especially toward the interior, are rather conspicuously silky-hairy and in that respect suggest var. *canus* (Benth.) B. & W. [W.H. Brewer & S. Watson]." Wilken (1993) did not recognize infraspecific taxa in *R. californicus* or *R. canus*, treating var. *ludovicianus* as a synonym of *R. canus*.

In preparations for publication of the second edition of the Vascular Plants of San Luis Obispo Co., California, I used the resources of the Consortium of California Herbaria database (CCH 2017) to access specimen records of plants collected in San Luis Obispo Co. Various specimen identifications came to light that I did not have in my records, and I sought to determine the accuracy of the determinations of these specimens. Among the reports was a collection by Frank Vasek in UCR determined as

Ranunculus canus var. *ludovicianus*. I requested a loan of this specimen and others from UCR, and upon examination of the specimen I determined it to be correctly identified according to the keys in the Jepson Manual (Whittemore 2012). This led me to the chronicle of the discovery of this plant in the 1880s, variations in its subsequent taxonomic disposition, the disappearance of its syntypes, and lecto- and neotypification that are recounted above. In April 2017 I set out to relocate Vasek's collection site and any other localities where the plants were growing, and my efforts resulted in finding plants from nearby sites that strongly resemble Vasek's specimen.

Achenes of *R. canus* var. *ludovicianus* were described by Benson (1948) as usually 4–5 mm. long, 3 mm broad; achenes of *R. californicus* were described as 1–2.8 mm long, 1–2.3 mm broad. In his key to species, Whittemore (2012) separated *R. canus* var. *ludovicianus* from *R. californicus* by "Fruit body 3.4–4.2 mm, beak deltate" vs. "Fruit body 1.8–3.2 mm, beak lanceolate." Fully mature achenes from Vasek's collection (from early May) measure up to 5 mm long and 4 mm wide. Immature achenes from the specimens I collected (in early April) had similar dimensions when fresh, but shrank somewhat in drying, the largest on individual dry specimens measuring 3.5–5 mm long and 3–3.5 mm wide. Otherwise similar plants from Shell Creek Road (Keil 34368, OBI) and La Panza Road (Keil 34394, OBI) had not-fully-filled achenes that measured within the size range for *R. canus* var. *ludovicianus* when fresh, but when dry the largest are 3 mm long, thus falling within the upper size range for *R. californicus*.

Significance. This report confirms the occurrence of the plant named by E. L. Greene as *Ranunculus ludovicianus* in its namesake county and pinpoints for the first time localities where these plants grow in the county. They are the first records of the taxon from San Luis Obispo Co. since the 1884 syntype collection by Katharine Curran.

Remaining to be investigated is the relationship between the San Luis Obispo Co. populations and the similar plants of the Tehachapi, San Bernardino, and San Diego areas which, since the studies of Benson (1941, 1948) have borne the name *R. canus* var. *ludovicianus*. Greene (1892) opined that the San Luis Obispo Co. plants should actually be treated as part of *R. californicus* in his var. *canescens*. But in the absence of any specimens from San Luis Obispo Co., Greene's hypothesis could not be investigated. Benson was unable to examine any San Luis Obispo Co. material ascribed to var. *ludovicianus* as a part of his studies. In the absence of specimens, and maybe also as a result of Greene's (1892) exclusion of the San Luis Obispo Co. plant, San Luis Obispo Co. disappeared from range statements in floristic treatments. Whittemore (1997) stated that "*Ranunculus canus* var. *ludovicianus* is endemic to the Transverse Ranges of California." But he expressed doubt as to

its placement in *R. canus*: “Pending further study, I am reluctantly following L. D. Benson’s (1948) placement of this taxon. The plants have the floral characters of *R. californicus* and have often been included in that species. Indeed, forms of *R. californicus* from the foothills west of the San Joaquin Valley may also have large achenes with deltate beaks. Those plants differ from *R. canus* var. *ludovicianus* only in their ovate leaf segments. Further study of the group is much needed.” [The shape of ultimate leaf segments of the San Luis Obispo Co. specimens varies from oblong-elliptic to lance-oblong.] Indeed the entire complex of *Ranunculus californicus*, *R. canus*, and *R. occidentalis* Nutt. is in need of an investigation using modern tools, but this is far beyond the scope of this report.

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DIFFERING PATTERNS OF GENETIC DIVERSITY AND INBREEDING IN TWO RARE SERPENTINE MONARDELLAS IN THE NORTHERN SIERRA NEVADA

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ABSTRACT

Monardella follettii (Jeps.) Jokerst and *M. stebbinsii* Hardham and Bartel are two rare endemic mints restricted to patchy ultramafic (serpentine) soil exposures in the northern Sierra Nevada. These species are rare because of small population sizes, low numbers of total populations, and limited availability of their specialized habitat. We collected samples from populations across the range of both species, and assessed genetic diversity, inbreeding, and genetic distance among populations. In the relatively more widespread *M. follettii*, we found low genetic diversity, little differentiation among populations, and no evidence of inbreeding. In contrast, we found significant inbreeding, higher genetic diversity, and high population differentiation over short distances in *M. stebbinsii*. We suggest continued protection and monitoring for *M. follettii*, but do not recommend any action intended solely for genetic management. To alleviate inbreeding in *M. stebbinsii*, we suggest pollen transfer from other conspecific populations.

Key Words: conservation, endemism, genetic diversity, genotyping by sequencing (GBS), inbreeding, *Monardella*, serpentine.

Small populations of endemic plants face an elevated risk of extinction from climate change and anthropogenic disturbance (Jump and Peñuelas 2005; Harrison 2013). With rising temperatures, plant populations are predicted to move higher in altitude and latitude, creating especially challenging circumstances for edaphic endemic plants with a low potential to shift their ranges if their specialized habitat is local and rare (Dullinger et al. 2012). These external threats faced by small plant populations may be compounded by the internal genetic challenges of inbreeding, low genetic diversity, and disruptive or insufficient amounts of gene flow that can reduce a population's ability to adapt (Ellstrand and Elam 1993). As land managers and conservationists strategize for the future, they can attempt to mitigate these genetic problems as part of multifaceted conservation plans for threatened plants.

Molecular population genetic analyses allow for the quantification of genetic diversity, inbreeding, and gene flow to elucidate the evolutionary histories and population dynamics of groups of populations. In population genetics, inbreeding appears as an increased level of homozygosity of a population, a state that has been shown to reduce fitness and increase extinction risk in experimental (Frankham 1995), natural (Saccheri et al. 1998), and simulated populations (O'Grady et al. 2006). As genetic diversity decreases, so too does the raw material on which evolution can act, potentially lowering population fitness in a changing environment (Reed and Frankham 2003). Gene flow among populations of a species can augment genetic variation and increase the fitness of a population low in genetic diversity

(Willi et al. 2007; Sexton et al. 2011). However, gene flow also can introduce maladaptive alleles and lower fitness if the populations are in divergent habitats (Fischer and Matthies 1997; Oakley et al. 2015). Conservation geneticists seek to leverage these genetic measures to guide management strategies for the preservation of rare and threatened species. In formulating management guidelines for conservation of endemic plants under sustained climate change, population genetic measures are an essential part of comprehensive conservation plans that include demographic assessments, a thorough understanding of an organism's ecology, and modeling of the geographic factors influencing a species' distribution (Schierenbeck 2017).

Monardella follettii (Jeps.) Jokerst and *M. stebbinsii* Hardham & Bartel are two rare, strict serpentine soil endemic mints in the northern Sierra Nevada of California. The species occur throughout the same serpentine soil belt, but their habitats are distinct. *Monardella stebbinsii* occurs on steep, exposed scree slopes and cliff ledges of serpentinite-derived soil in a small geographic range around the mountain known as Red Hill. Populations of *M. follettii* occur on less extreme slopes of peridotite-derived soil across a larger range in Lassen and Plumas Counties (Coppoletta and Woolhouse 2010). The plants are easily morphologically distinguishable. *Monardella follettii* exhibits a mat-like growth and pale-green leaves, whereas *M. stebbinsii* grows as a subshrub with purple-green leaves densely covered in glandular hairs. The reproductive structures of the species are similar in size, phenology, and color, but *M. stebbinsii* has larger inflorescences with more

flowers (Sanders et al. 2013). Both species exhibit significant decreases in seed production when prevented from outcrossing, and *M. stebbinsii* shows lower seed set than *M. follettii*, despite having more pollinator visits (Woolhouse 2012). *Monardella stebbinsii* is reported to be a diploid of $n = 21$ with some individuals occasionally exhibiting aneuploidy (Hardham and Bartel 1990). No chromosome counts have been explicitly reported for *M. follettii*; however the base number of chromosomes in *Monardella* is thought to be $n = 21$ (Raven et al. 1965; Hardham and Bartel 1990).

The patchy, limited habitat restricts both species to a small number of populations across Plumas and Lassen National Forests. This equates to about 15 populations and fewer than 1500 individuals of *M. stebbinsii* and 25 populations and 5000–10,000 individuals of *M. follettii*. The California Native Plant Society lists both plants as status 1B.2, signifying moderate threats to 20–80% of the populations. NatureServe ranks *M. stebbinsii* and *M. follettii* as status G2 and G1, respectively, because of the small numbers of populations and individuals. The United States Forest Service, which manages most of the land on which these species occur, lists both taxa as critically imperiled. The loss of a single population could be a major detriment to the survival of the species.

In addition to the genetic consequences of small population size, these species face a number of anthropogenic threats, including logging operations, increasing frequency of wildfires, firefighting activities (e.g., the construction of fire lines), and road construction. Populations of *M. stebbinsii* are especially susceptible to erosion and anthropogenic disturbance because of the steep habitat. A recent conservation assessment recommends that people take extreme care when surveying *M. stebbinsii*, as a minor disturbance can cause the soil underneath the plants to completely slide away (Woolhouse 2012). Further, both species are difficult to propagate in the greenhouse and growing large numbers of individuals for restoration would be challenging.

Phylogenetic relationships within *Monardella* are poorly understood, but some have hypothesized relationships based on morphology and geographic distribution. Elvin and Sanders (2009) placed *M. stebbinsii* in an alliance of relictual mountaintop species with similar morphology. However, Hardham and Bartel (1990) argued that *M. stebbinsii* is not closely related to any other member of the genus. Elvin and Sanders (2009) further suggested *M. follettii* belongs in the *Odoratissimae* alliance defined by glabrous leaves and a suffrutescent habit. The historic population sizes of these species, their progenitors and the extent to which they are reproductively isolated are unknown. However, these factors may influence the amount of genetic diversity in populations, as leaky reproductive barriers could allow for gene flow between these two species and other nearby members of *Monardella*.

Here we present the results of a population genetic survey of the two rare, serpentine-endemic *Monardella* species of Plumas and Lassen National Forests and use the data to construct conservation recommendations for land managers. We sample plants from six populations of *M. follettii* and four populations of *M. stebbinsii*, representing the range of both species through Plumas and Lassen National Forests. We use a genotyping by sequencing (GBS) approach to discover single nucleotide polymorphisms (SNPs) in each species and estimate population genetic parameters. We ask to what extent populations of the two species are genetically diverse, isolated from each other across the landscape, and/or inbred. We then synthesize management strategies to guide assisted gene flow and seed banking.

METHODS

Collections

We collected plant tissue from six *M. follettii* and four *M. stebbinsii* Forest Service-described occurrences (hereafter “populations”) from across Plumas and Lassen National Forests, spanning the entire geographic range of both species (Table 1, Fig. 1). We chose our collection sites to match previous work completed as part of an ecological and demographic assessment of the two taxa (Coppoletta and Woolhouse 2010). These populations are abbreviated with the prefixes “MOFO” for *M. follettii* and “MOST” for *M. stebbinsii*, and we have adopted this Forest Service nomenclature for consistency. MOFO3003, MOST005, and MOST003 have Forest Service suboccurrences that were lumped into single populations, as we assumed they were close enough to allow frequent and consistent gene flow. These suboccurrences spanned up to 200 m for MOFO3003 and <15 m for the *M. stebbinsii* populations. At each site, we sampled small amounts of leaf or flower bud tissue from 20–30 individuals, or fewer samples that represented every individual in the population. We vouchered entire plants from most populations for which herbarium accessions did not exist, but did not harvest from populations with very small numbers of individuals. We deposited the specimens into the herbarium at the University of California, Santa Cruz (UCSC).

DNA Extractions

We extracted total DNA (i.e., nuclear, plastid, and mitochondrial DNA) from all individuals using a modified CTAB protocol (Doyle and Doyle 1987). We tested all DNA samples for purity with a NanoDrop spectrophotometer (ThermoFisher Scientific, Wilmington, Delaware), evaluated degradation and shearing with agarose gel electrophoresis, and quantified concentrations with a Qubit fluorometer (Invitrogen, Carlsbad, California). For samples that were insufficiently clean or slightly degraded, we

TABLE 1. *MONDARDELLA* COLLECTION LOCATIONS. GPS coordinates in WGS84 of locations of the populations sampled in this study. Population names correspond to Coppoleta and Woolhouse (2010) and Woolhouse (2012). Because of very small population sizes, herbarium specimens were only taken if existing accessions did not exist and sufficient individuals were present in the population.

Population	Species	Latitude	Longitude	Altitude (m)	Herbarium accession
LFO	<i>M. follettii</i>	40.085904	−121.276602	1347	UCSC8318
MOFO 3009	<i>M. follettii</i>	39.924422	−121.033577	1219	UCSC8316
MOFO 3005	<i>M. follettii</i>	39.925070	−121.077590	1158	JEPS63417
MOFO 3003	<i>M. follettii</i>	40.050508	−121.236500	1463	UCSC8310
MOFO 3002	<i>M. follettii</i>	40.043186	−121.179569	1767	CAS886559
MOFO 3001Nn	<i>M. follettii</i>	39.991851	−121.105293	1584	UCSC8312
MOST 005	<i>M. stebbinsii</i>	40.013566	−121.192917	792	UCSC8311
MOST 004	<i>M. stebbinsii</i>	40.046765	−121.218556	822	CHSC34000
MOST 003	<i>M. stebbinsii</i>	40.023904	−121.166290	853	UCSC8314
MOST 001	<i>M. stebbinsii</i>	40.052640	−121.208145	762	N/A

further cleaned the extractions with a sodium acetate-ethanol precipitation protocol. We chose to genotype the twenty individuals from each population with the highest DNA quality. Once we had genotyped our samples, we simulated population genetic analyses under different numbers of individuals and genetic markers with SPOTG, a conservation genetics planning tool (Laval and Excoffier 2004; Excoffier and Lischer 2010; Hoban et al. 2013),

and found we sampled appropriately with sufficient marker numbers, individuals, and populations for robust analysis.

Library Construction and Sequencing

We sent DNA samples to the Institute for Genomic Diversity at Cornell University (Ithaca, New York) for GBS library construction (Elshire et

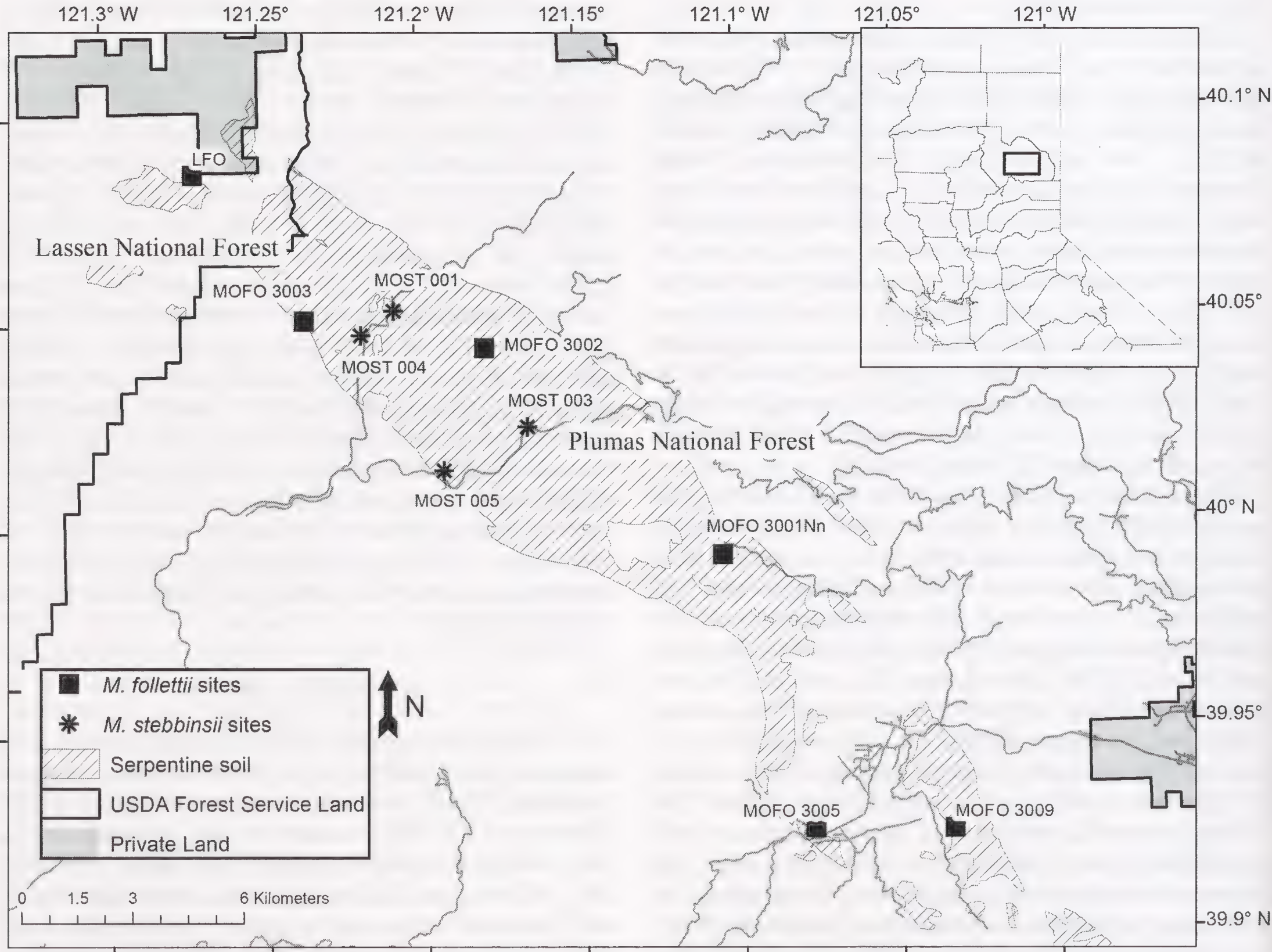


FIG. 1. Map of the study area showing all populations sampled. Species are represented by different shapes. Inset shows expanded map of northern California with a box indicating the approximate area shown in the main map.

al. 2011) and Illumina HiSeq 2500 sequencing (San Diego, California). To construct the libraries (i.e., collections of DNA fragments with known sequences to facilitate sequencing), individual DNA samples were digested with the restriction enzyme *Pst*I, barcoded with adapters of known sequence, and pooled into groups of ninety-five individuals and one negative control. Next, each library was amplified by polymerase chain reaction (PCR) and sequenced in a single lane, returning about two hundred million 100-base-pair sequences per library.

Sequence Analyses and SNP Calling

We analyzed the raw sequence data with the TASSEL/UNEAK bioinformatics pipeline to generate biallelic SNP calls from the raw sequence data (Bradbury et al. 2007; Lu et al. 2013a). Briefly, the Universal Network Enabled Analysis Kit (UNEAK) pipeline sorts the raw sequences by individual barcode, trims the sequences to 64 base pairs, compiles exactly matching reads as tags, aligns the sequences among individuals to find tags differing at only one base, creates networks of these nearly matching tags, and filters networks that are too complex (i.e., tags with too many SNPs). We employed strict sequence-quality filtering parameters and a minimum coverage threshold of 3 to call a SNP (Lu et al. 2013b). We ran the pipeline separately for each species to maximize the number of loci suitable for within-species analysis. We removed individuals missing more than 20% of the data and loci that were not present in at least 80% of individuals. After this process, we obtained for each species a final matrix that contains a row for each individual with columns for its nucleotide identity at a given locus.

Population Genetic Analyses

To understand genetic diversity and inbreeding within species, we calculated summary statistics for each species using the software GenAlEx 6.5 (Peakall and Smouse 2012). These summary statistics include the number of private alleles, private allele frequency, expected heterozygosity (H_E), and observed heterozygosity (H_O). Expected heterozygosity indicates the genetic variability in a population, and the difference between expected and observed values indicates inbreeding. The count and frequency of private alleles give a simple indication of the extent of gene flow between populations. We derived the summary statistics per individual and averaged across all loci. We tested for significant differences between H_E and H_O with Bartlett tests of homogeneity of variances in the *ade4* (Jombart and Ahmed 2011) and *stats* (R Core Team 2016) packages in R.

To evaluate genetic structuring among individuals and populations, we assigned individuals to genetic clusters based on similarities in patterns of genetic variation with a Bayesian assignment analysis implemented in the software STRUCTURE (Pritch-

ard et al. 2000). For both datasets, we ran STRUCTURE using the admixture model with 50,000 burn-in steps, which allows the algorithm to stabilize around realistic values before collecting data from simulations, followed by 100,000 steps. We estimated the hyperparameter λ for each dataset before running the simulations, and subsequently fixed it at the estimated value, as suggested for SNP data sets by Pritchard et al. (2000). For the *M. follettii* and *M. stebbinsii* data sets, we set the prior for most likely number of clusters (K) as 1–6 and 1–4, respectively, based on the number of populations sampled. We ran fifteen replicates for each K . We examined the rate of change of probability in successive numbers of K to determine the most likely number of genetic clusters for each species (Evanno et al. 2005) with the software STRUCTURE HARVESTER (Earl and von Holdt 2012).

We used Analysis of Molecular Variance (AMOVA) and F-statistics to further examine heterozygosity at the individual, population, and species level. We ran a locus-by-locus AMOVA (Excoffier et al. 1992) using the codominant allelic input with 9999 permutations, and calculated pairwise F-statistics with 999 permutations for significance testing (Wright 1969). We corrected P-values for multiple comparisons for F-statistics using a Holm-Bonferroni adjustment (Holm 1979). F_{IS} , F_{ST} , and F_{IT} make up the F-statistics and are known as the inbreeding coefficient, fixation index, and overall fixation index, respectively. F_{IS} represents the reduction of heterozygosity of a population due to inbreeding, F_{ST} represents the differentiation among populations as a reduction in heterozygosity due to genetic drift within a population, and F_{IT} represents the total expected reduction in heterozygosity of an individual, i.e., the sum of F_{IS} and F_{ST} . We chose to interpolate missing data in the calculation of F-statistics to avoid biased sources of variation.

In order to visualize genetic distances among individuals and how those relate to population membership, we calculated codominant genetic distances among individuals (Peakall et al. 1995) and summarized the results in a principal coordinates analysis (PCoA). Like a principal components analysis (PCA), a PCoA is a method to summarize multivariate data, but a PCoA looks for dissimilarities in the data set as opposed to the similarities sought in a PCA. Finally, we tested for isolation by distance using a paired Mantel Test of linearized pairwise F-statistics and their corresponding pairwise geographic distances to determine if populations that are geographically farther from each other are more genetically distinct than populations closer to each other.

RESULTS

SNP Calling

The Illumina sequencing returned about 600 million reads for *M. stebbinsii* and *M. follettii*. After

TABLE 2. SUMMARY STATISTICS (MEAN \pm SE) FOR *MONARDELLA FOLLETTII*. Population names correspond to Coppoleta and Woolhouse (2010) and Woolhouse (2012).

	Population						Mean
	MOFO 3001Nn	MOFO 3 002	MOFO 3003	MOFO 3005	MOFO 3009	LFO	
Observed heterozygosity (H_O)	0.124 (± 0.009)	0.157 (± 0.01)	0.181 (± 0.011)	0.157 (± 0.01)	0.135 (± 0.009)	0.153 (± 0.010)	0.151 (± 0.004)
Expected heterozygosity (H_E)	0.136 (± 0.008)	0.149 (± 0.008)	0.160 (± 0.008)	0.149 (± 0.008)	0.135 (± 0.008)	0.149 (± 0.008)	0.146 (± 0.003)
Private allele frequency	0.005 (± 0.004)	0.008 (± 0.005)	0.008 (± 0.005)	0.011 (± 0.005)	0.005 (± 0.004)	0.008 (± 0.005)	
Number of private alleles	2	3	3	4	2	3	

filtering for quality and coverage, we identified 675 SNP loci in 78 individuals and 365 SNP loci in 100 individuals for the *M. stebbinsii* and *M. follettii* data sets, respectively. Before filtering, these totals were 5693 loci and 3318 loci in *M. stebbinsii* and *M. follettii*, respectively.

Genetic Diversity

Populations of *M. follettii* exhibit a mean H_E of 0.146, and values are consistent across the range of the species (Table 2). Populations of *M. stebbinsii* show a mean H_E of 0.209 and a significantly lower mean H_O of 0.165 (Bartlett test of homogeneity of variances, $P < 0.001$) (Table 3), a pattern indicative of genetic drift or inbreeding. Private allele frequencies, which indicate the extent to which populations have differentiated, are about four times higher in *M. stebbinsii* compared to *M. follettii*.

Genetic Structure

The assignment of individuals in the STRUCTURE analysis reveals how the populations cluster based on genotypes alone. Using the ΔK method to examine our STRUCTURE analysis, we find $K = 3$ to be the most likely number of clusters for *M. follettii* (Fig. 2) and $K = 2$ to be the most likely number of clusters for *M. stebbinsii* (Fig. 3). In *M. follettii*, all individuals are assigned mainly to one major cluster. After this first major assignment, the populations vary based on their assignment to one of the remaining two clusters, with individuals within each population showing fairly consistent assignment to the same proportion of the same clusters. For *M. stebbinsii*, MOST004 and MOST001 individuals

show assignment almost entirely to one of the two genetic clusters, and MOST005 exhibits assignment to the other genetic cluster. The fourth population, MOST003, splits across both clusters, and individuals generally show more variable assignment than individuals in other populations. Clustering in *M. stebbinsii* mirrors the geographic locations of the populations, with MOST001 and MOST004 geographically close together on the northwest side of Red Hill whereas MOST003 and MOST005 are separated from each other by a few kilometers on the south side of Red Hill.

AMOVA and F-statistics reveal how genetic variation is partitioned across the hierarchical levels of individual and population. In *M. follettii*, among-population differences are only responsible for 2% of the genetic variation, and the remaining 98% arises from within individuals (Table 4). The slight negative numbers simply indicate a lack of genetic structure at this level (Excoffier 2000). The overall F_{ST} values indicate very little genetic structure among populations of *M. follettii* (Table 5), and only one pairwise F_{ST} (MOFO3002 & MOFO3009) is significant ($F_{ST} = 0.038$, $P < 0.05$ after Bonferroni adjustment). These low F_{ST} levels suggest little differentiation among populations of *M. follettii*. In *M. follettii*, F_{IT} and F_{IS} , do not differ significantly from zero, suggesting little inbreeding in the populations. The *M. stebbinsii* data show 8% of variation among populations, and the rest of the variation partitioned within and among individuals (Table 6). In contrast with *M. follettii*, we find significant structure among populations in *M. stebbinsii* with an overall F_{ST} of 0.082 (Table 7). Moreover, *M. stebbinsii* exhibits a large, significant F_{IS} of 0.210, suggesting substantial inbreeding across the species. All pairwise F_{ST}

TABLE 3. SUMMARY STATISTICS (MEAN \pm SE) FOR *MONARDELLA STEBBINSII*. Population names correspond to Coppoleta and Woolhouse (2010) and Woolhouse (2012).

	Population				Mean
	MOST 001	MOST 003	MOST 004	MOST 005	
Observed heterozygosity (H_O)	0.17 (± 0.007)	0.159 (± 0.006)	0.15 (± 0.006)	0.179 (± 0.007)	0.165 (± 0.003)
Expected heterozygosity (H_E)	0.208 (± 0.007)	0.218 (± 0.007)	0.202 (± 0.007)	0.208 (± 0.007)	0.209 (± 0.003)
Private allele frequency	0.053 (± 0.009)	0.03 (± 0.007)	0.037 (± 0.007)	0.012 (± 0.004)	
Number of private alleles	36	20	25	28	

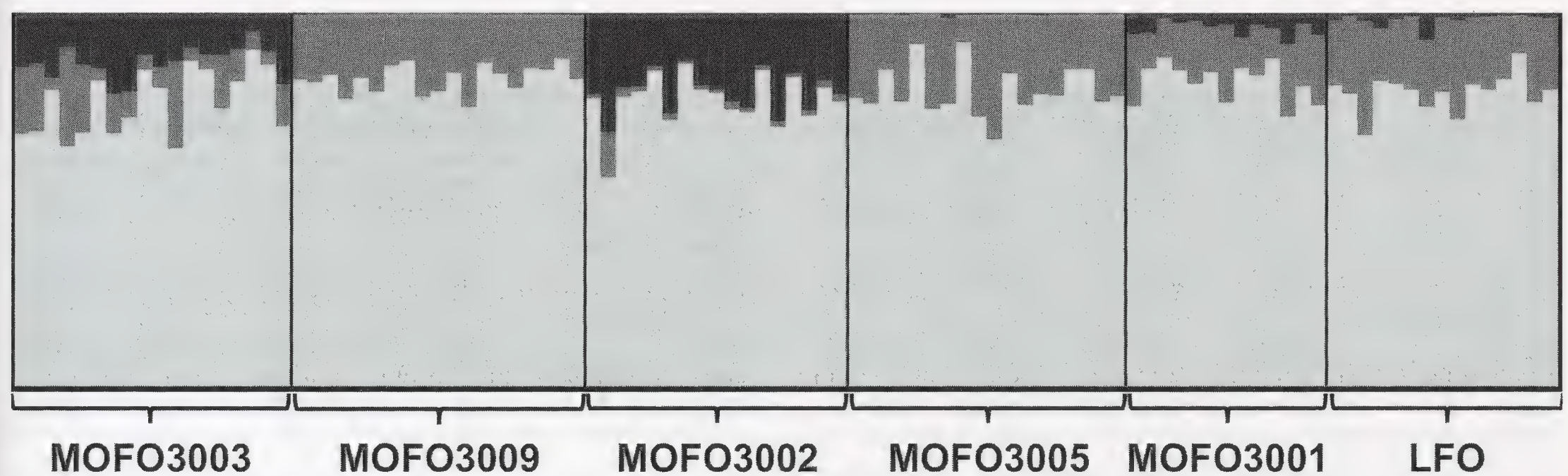


FIG. 2. Assignment of *M. follettii* individuals to genetic clusters as determined by the software STRUCTURE. Each stacked bar represents one individual and different shades show the proportion of its assignment to each of $K = 3$ genetic clusters. Population names are noted below groups of bars. All individuals are mostly assigned to one cluster with populations differing based on their assignment to the second or third cluster.

comparisons are significant in *M. stebbinsii* (Table 8), and their geographic pattern mirrors the cluster assignment of the STRUCTURE analysis. Mantel tests for isolation by distance using linearized pairwise F_{ST} values were not significant for either species.

The principal coordinate analyses show how individual pairwise genetic distances correlate with population identity. Our analyses mirror patterns of genetic structure as determined by STRUCTURE and F_{ST} . In *M. follettii*, we see little discernable clustering of individuals in populations in the first two coordinates (Fig. 4), suggesting that genetic distances between individuals are not correlated with an individual's geographic location. Thus little differentiation seems to have occurred at the population level in *M. follettii*. The MOST001 and MOST004 populations of *M. stebbinsii* show tight clustering on the first coordinate and some differentiation along the second coordinate (Fig. 5). About half of the individuals from MOST003 cluster tightly with MOST001 and MOST004, but the remaining individuals are highly differentiated from MOST001 and MOST004 on the first coordinate and spread

diffusely on the second coordinate. Individuals in MOST005 show the same pattern as this latter half of the MOST003 individuals.

DISCUSSION

We set out to determine the extent of inbreeding, the level of genetic diversity, and the connectedness of populations of two rare, serpentine-endemic *Monardella* species in the northern Sierra Nevada. We found different population genetic patterns in the two species. In *M. follettii* we found no evidence of inbreeding within populations with H_E and H_O of 0.146 and 0.151, respectively, and little differentiation among populations. Populations of *M. stebbinsii* showed significant inbreeding within populations with H_E and H_O of 0.209 and 0.165, respectively, and significant population differentiation over very short distances. These differing patterns tell two different stories for these two, rare congeners. *Monardella follettii*, the species with greater numbers of individuals and populations, appears to have lower genetic diversity as quantified by H_E than the very rare, restricted *M. stebbinsii*. Though *M.*

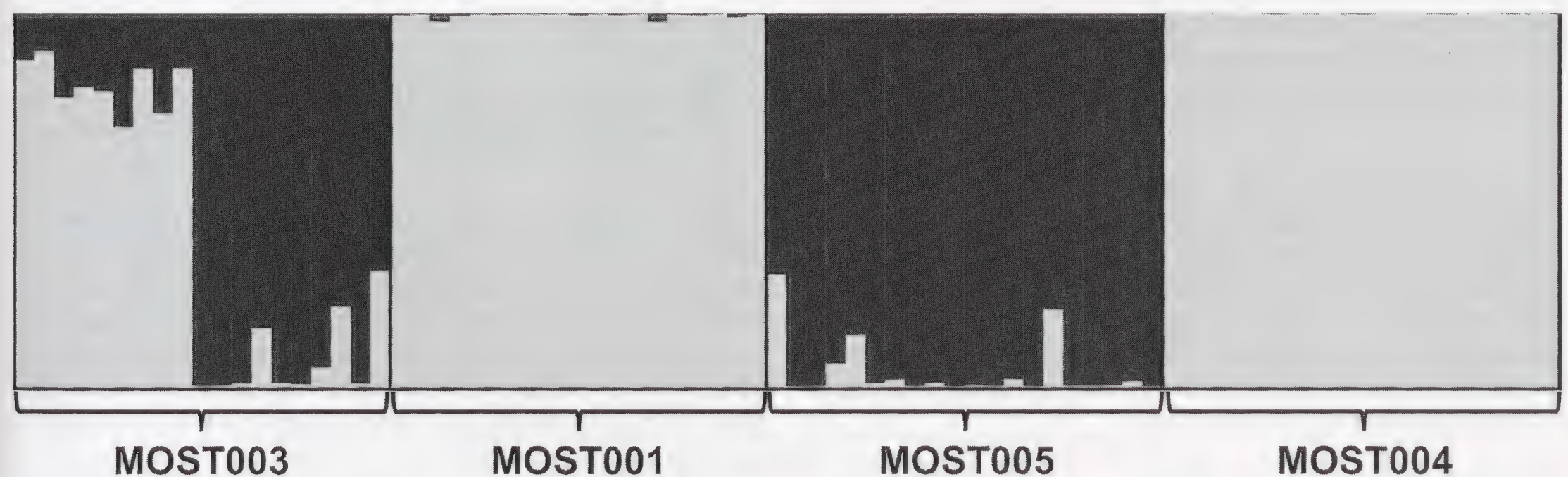


FIG. 3. Assignment of *M. stebbinsii* individuals to genetic clusters as determined by the software STRUCTURE. Each stacked bar represents one individual and different shades show the proportion of its assignment to each of $K = 2$ genetic clusters. Three of four populations are assigned almost entirely to one or the other cluster. The fourth population splits assignment between the two clusters.

TABLE 4. ANALYSIS OF MOLECULAR VARIANCE (AMOVA) FOR *MONARDELLA FOLLETTII*.

Source of genetic variation	Degrees of freedom	Sum of squares	Estimated variance	Percent variance
Among Populations	5	225.714	0.584	2.1%
Among Individuals	94	2323.238	−0.098	−0.3%
Within Individuals	100	2552.000	27.796	98.3%
Total	199	5100.952	28.283	100%

stebbinsii has a higher measure genetic diversity, its populations exhibit extensive inbreeding. Further, populations of *M. stebbinsii* appear to have differentiated to a much greater extent than populations of *M. follettii*, despite its much smaller range and shorter distances between populations. Below, we examine each of these patterns in more depth and offer conservation recommendations based on our interpretations of these data.

Patterns of genetic diversity can elucidate recent and past evolutionary histories of the species under examination, and our data tell diverging stories for *M. stebbinsii* and *M. follettii*. To understand the levels of H_E in the context of other plants, it is important to know that H_E varies based on molecular marker type, and SNPs generally show lower diversity than microsatellites (e.g., Ryyänen et al. 2007). Using GBS to evaluate rare plant populations remains a new area of conservation genetics, and few data sets exist to compare our data to other similar species using these markers. From the available data, large populations of angiosperms typically exhibit H_E in the range of 0.18–0.30 when examined with SNP markers (e.g., Vandepitte et al. 2012; Saxena et al. 2014; Schilling et al. 2014). Levels of H_E in populations of *M. stebbinsii* fit in this range, suggesting the rare species has genetic diversity equal to species with many times the numbers of populations and individuals. The level of genetic diversity in *M. stebbinsii* could mean the species was once more widespread, and it has only recently become rare. Such events are known as population bottlenecks and eventually result in very low H_E as alleles are lost to genetic drift (Young et al. 1996). Although *M.*

TABLE 5. ANALYSIS OF MOLECULAR VARIANCE (AMOVA) F-STATISTICS FOR *MONARDELLA FOLLETTII*. P-values determined by randomization. F_{IS} represents the reduction of heterozygosity of a population due to inbreeding, F_{ST} represents the differentiation among populations as a reduction in heterozygosity due to genetic drift within a population, and F_{IT} represents the total expected reduction in heterozygosity of an individual.

F-statistic	Value	P-value
F_{ST}	0.021	<0.001
F_{IS}	−0.012	0.745
F_{IT}	0.010	0.292

TABLE 6. ANALYSIS OF MOLECULAR VARIANCE (AMOVA) FOR *MONARDELLA STEBBINSII*.

Source of genetic variation	Degrees of freedom	Sum of squares	Estimated variance	Percent variance
Among Populations	3	1024.598	6.495	8%
Among Individuals	74	6532.429	15.315	19%
Within Individuals	78	4496.366	57.646	73%
Total	155	12053.393	79.456	100%

stebbinsii is currently restricted to a rare habitat type, this could be through paleoendemism, in which a once widespread species becomes relegated to marginal habitat, such as serpentine barrens, because of low competitive ability (Raven and Axelrod 1978). We would need a resolved phylogeny to evaluate this hypothesis, given that there is no detailed fossil record. *Monardella follettii* shows lower H_E despite greater numbers of individuals and a more widespread distribution of habitat and populations. In general, low H_E as seen in *M. follettii* is concerning for rare, threatened plants, but it is not necessarily unexpected given the small population sizes in these species (Ellstrand and Elam 1993; Paschke et al.2002).

A primary goal of this study was to quantify genetic diversity in order to strategize for the maintenance of evolutionary potential of *M. follettii* and *M. stebbinsii*. Only *M. follettii* shows low H_E compared to populations of other species of widespread angiosperms (Gitzendanner and Soltis 2000). A paucity of genetic diversity can increase a population’s susceptibility to a number of extinction-inducing events. Low genetic diversity can decrease a population’s resistance to disease and may leave it less capable of persisting through rapid or sustained changes in climate or other environmental conditions (Spielman et al. 2004; Jump et al. 2009), though it is impossible to directly relate our measures of neutral genetic variation to adaptive genetic variation (Holderegger et al. 2006).

Under current climate projections, populations of both species will have to either adapt to higher temperatures or migrate higher in elevation or northward to track their current climate envelope.

TABLE 7. F-STATISTICS FOR *MONARDELLA STEBBINSII*. P-values determined by randomization. F_{IS} represents the reduction of heterozygosity of a population due to inbreeding, F_{ST} represents the differentiation among populations as a reduction in heterozygosity due to genetic drift within a population, and F_{IT} represents the total expected reduction in heterozygosity of an individual.

F-statistic	Value	P-value
F_{ST}	0.082	<0.001
F_{IS}	0.210	<0.001
F_{IT}	0.274	<0.001

TABLE 8. PAIRWISE F_{ST} VALUES FOR *MONARDELLA STEBBINSII* POPULATIONS. Population names correspond to Coppoleta and Woolhouse (2010) and Woolhouse (2012). Asterisks indicate significance at $P < 0.01$.

	MOST 001	MOST 003	MOST 004	MOST 005
MOST 001				
MOST 003	0.073*			
MOST 004	0.069*	0.071*		
MOST 005	0.118*	0.026*	0.125*	

Uphill migration potential for some populations of *M. follettii* may be limited, because many populations are already located along ridges and mountaintops. Likewise the steep slopes of *M. stebbinsii* habitat may make upward migration to higher altitudes difficult for the species, and the genetic structure seen over short distances suggests that *M. stebbinsii* generally does not disperse even small distances. Looking forward over many decades, both species may face severe challenges from climate change. *Monardella stebbinsii* and *M. follettii* occur in some of the northernmost mountains in the Sierra Nevada, and the volcanic bedrock of the Cascades to the north does not give rise to many serpentine soils. With assisted migration there may be suitable habitat in the Klamath Mountains, but these *Monardella* exhibit fine-scale partitioning in their specific serpentine habitats and may not survive in other serpentine soils (Woolhouse 2012; Kay et al. unpublished data). In addition to human disturbance, climate change, and the genetic challenges of small population sizes, *M. follettii* is threatened by wildfire. Several wildfires have scorched populations of *M. follettii* over the last 15 yr, including the LFO and MOFO3003 populations described here. Wildfires are expected to increase in frequency under sustained climate change towards a warmer and drier climate (Westerling et al. 2006), and even fire-adapted species can be suscep-

tible to a low fire-return interval that exhausts seed banks and allows establishment of competing invasive species (Whisenant 1990; Jacobsen et al. 2004). In outbreeding plants, low genetic diversity can reduce viable seed set when fewer unrelated mates are available (Byers and Meagher 1991; Young and Pickup 2010). Though mechanisms for self-incompatibility remain unknown for *Monardella*, our focal species are visited by a wide variety of insect pollinators (primarily bees) and show large decreases in seed set when pollinators are prevented from accessing the flowers in the field. Compared to open-pollinated controls, bagged inflorescences of *Monardella follettii* and *M. stebbinsii* show 75 and 73 percent lower seed set, respectively (Woolhouse 2012). Moreover, the open-pollinated control inflorescences show much lower seed set for the relatively inbred *M. stebbinsii* (45%) compared to *M. follettii* (77%), even though pollinator visitation is slightly higher, suggesting that *M. stebbinsii* may be having problems accessing appropriate mates (Woolhouse 2012). High homozygosity and significant F_{IS} indicate significant inbreeding throughout *M. stebbinsii* that does not occur in *M. follettii*. The sustained inbreeding and persistently small population sizes in *M. stebbinsii* likely reduce fitness through the accumulation of deleterious mutations in populations of the species (Frankham 1995). The larger population sizes of *M. follettii*, usually hundreds of individuals compared with tens of individuals in *M. stebbinsii*, likely enable more random mating in *M. follettii*. In conservation planning it is important to understand population genetic structure, which reveals the interpopulation dynamics of a species. Such information can indicate which populations may be examined for local adaptation before conservation action is taken (McKay et al. 2005). The two rare *Monardella* species of Plumas and Lassen National Forests exhibit different patterns of genetic structure. The STRUCTURE, PCoA, and

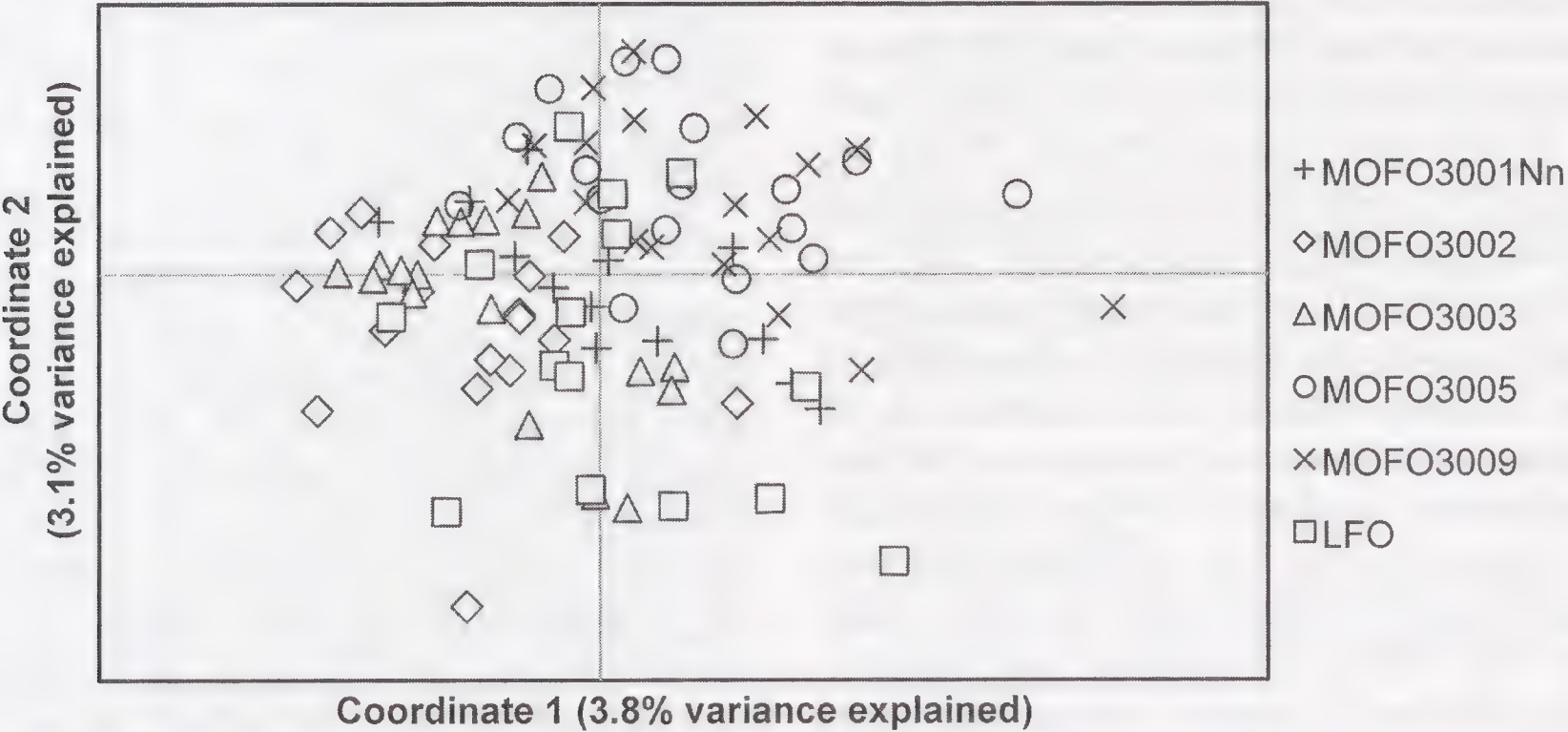


FIG. 4. Principal coordinates analysis of genetic distances for all individuals of *M. follettii*. Individuals are positioned in space according to the first two coordinates from a summarized transformation of a pairwise genetic distance matrix. Little separation between the populations is evident, with only MOFO3002 and MOFO3009 showing no immediate overlap. Coordinate 3 (not shown) explained an additional 2.9% of the variation.

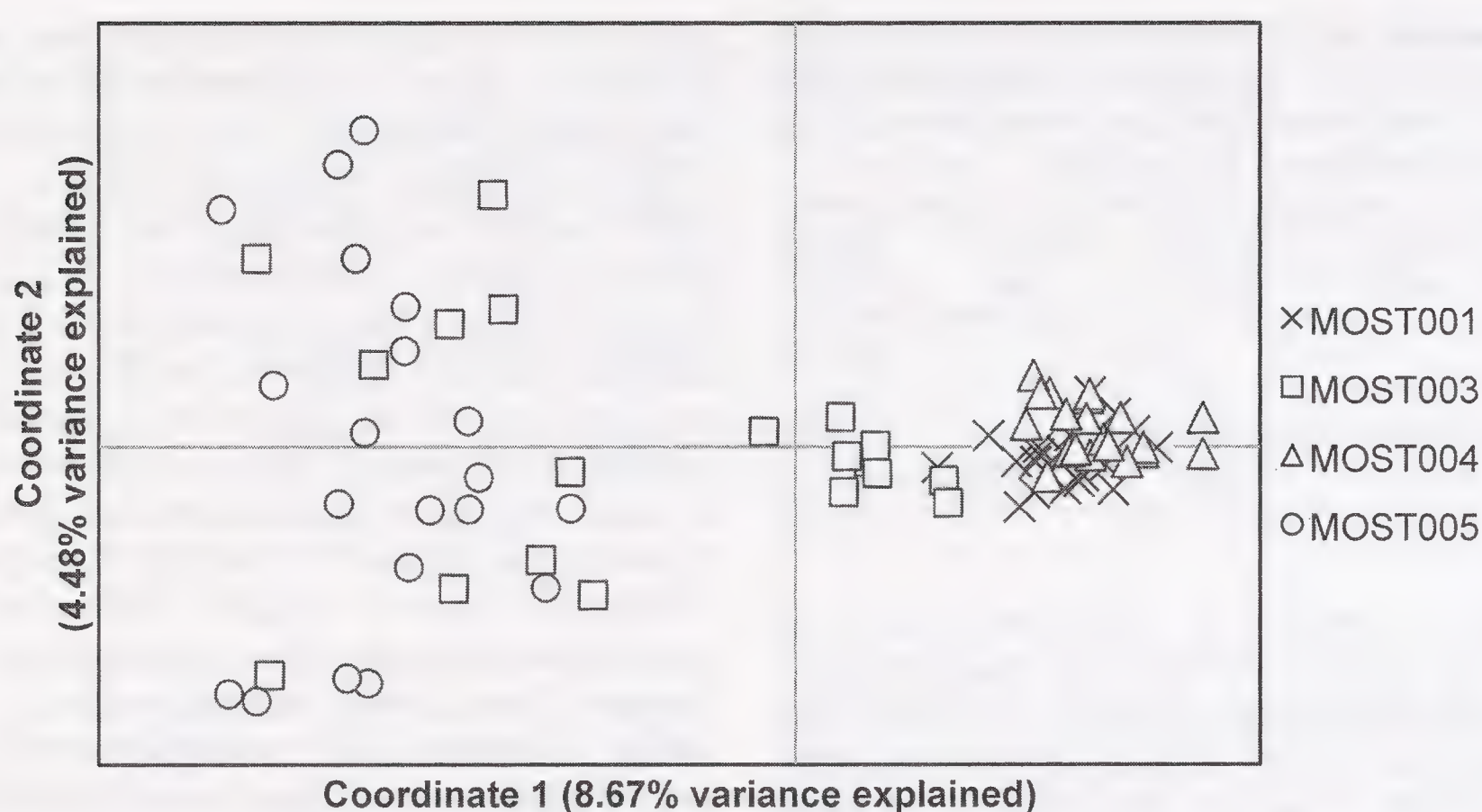


FIG. 5. Principal coordinates analysis of genetic distances for all individuals of *M. stebbinsii*. Individuals are positioned in space according to the first two coordinates from a summarized transformation of a pairwise genetic distance matrix. MOST001 and MOST 004 overlap almost entirely in the first and second coordinate, with almost no overlap of the other two populations. Coordinate 3 (not shown) explains an additional 4.12% of the variation.

F_{ST} analyses reveal little genetic structure among populations of *M. follettii* suggesting frequent gene flow or little separation in time between populations. Conversely, our results show little gene flow and high structure between *M. stebbinsii* populations, even though they occur around a single mountain. In small populations, genetic drift can rapidly fix alleles and contribute to differentiation among populations. Others have shown F_{IS} to be correlated with genetic structure among populations (Barrett and Kohn 1991; Duminil et al. 2007) suggesting the patterns seen in *M. stebbinsii* likely result from a combination sustained inbreeding and rapid genetic drift, as opposed to long time periods of isolation. In contrast, the larger populations of *M. follettii* populations do not exhibit any detectable inbreeding. Differences in the habitat may further explain some of the differing patterns seen in the two species, as *M. stebbinsii* inhabits a much rarer, patchier, and more extreme landscape than *M. follettii*.

Prior to this conservation assessment, no genetic resources had been developed for *Monardella*, and GBS proved to be an effective choice. With no genomic resources, we were able to develop hundreds of sequence-based markers for each species. Still, the number of SNPs derived from our analysis was lower than anticipated given the thousands described in other systems (Allendorf et al. 2010; Elshire et al. 2011). The lower numbers in our system may be due in part to incomplete digestion of the *Monardella* DNA, which tended to be full of terpenoids and other secondary plant metabolites, despite many refinements to the DNA extraction and cleanup protocols. The difference in the amount of SNPs between the two species is likely in part a result of generally lower genetic variation in *M. follettii* as the TASSEL/UNEAK pipeline requires polymorphism to call a locus. Nevertheless, the number of SNPs in

our analysis were more than sufficient to quantify genetic diversity, inbreeding, and structure of the two species.

Conservation Considerations

Monardella stebbinsii and *M. follettii* are two of the rarest plant species in Plumas and Lassen National Forests, and the genetic parameters derived from this study can inform management policy. Our recommendations assume that genetic diversity is essential for the long-term evolutionary potential of the populations (Honnay and Jacquemyn 2007), inbreeding can increase extinction risk (O'Grady et al. 2006), and maximizing genetic diversity through transplantation should not compromise local adaptation (McKay et al. 2005). *Monardella* congeners likely hybridize (Sanders et al. 2013; Kay unpublished data), so we advise caution in moving genetic material from any population in which two species of *Monardella* co-occur. However, these two rare species of concern exhibit very different specialized habitats and may be unable to grow in each other's habitats (Woolhouse 2012).

With little inbreeding, high apparent gene flow, and approximately equal genetic diversity in most populations, *Monardella follettii* would likely not benefit from genetic supplementation via pollen or seed movement. Likewise, low genetic distance among populations indicates managers likely do not need to be concerned with the source of plant material if a catastrophic event requires reestablishing a population. In some species in wildfire-prone environments, fire induces seed germination and recruitment of new individuals, which might reveal genetic diversity in a dormant seed bank (Menges and Dolan 1998). Conversely, frequent burn can act as a bottleneck that greatly reduces a population's size and encourages genetic drift and a resulting low

genetic diversity (England et al. 2002). Of the two species examined, *M. follettii* populations are more likely to burn given their more densely occupied habitat. No simulated burn or scarification is needed to germinate seeds in the greenhouse, therefore it is unlikely that the plants require wildfire for recruitment (Woolhouse 2012). In *M. follettii* populations that have recently burned (e.g., MOFO3003, LFO), we found no evidence fire has negatively or positively influenced genetic diversity compared to populations that have not recently burned. Therefore, we cannot recommend the use of artificial burn as a management strategy for *M. follettii* to increase genetic diversity. However, we also think large populations that have not recently burned will not be greatly affected by artificial burns designed to manage other co-occurring species. Ultimately we do not think this species would benefit from any strategies targeted at genetic management, but populations will surely benefit from continued protection from human and environmental threats.

The high inbreeding coefficient in *M. stebbinsii* suggests the species could benefit from population genetic management. Pollen movement could likely relieve inbreeding in populations of *M. stebbinsii*. In the scarlet gilia (*Ipomopsis aggregata*, Polemoniaceae) pollen transfers resulted in increased seed size and count in small, inbred populations (Heschel and Paige 1995). Such transfers would need to be undertaken carefully, since the *M. stebbinsii* habitat is very fragile, and even careful walking around these plants can cause extensive erosion. Therefore, we suggest managers place inflorescences from disparate populations in vases at the periphery of the steep, unstable *M. stebbinsii* habitat, in a location that will not cause erosion. Due to the very low population sizes in *M. stebbinsii*, we suggest managers only take inflorescences from a small number of individuals, as any clipping of flowers represents a substantial reduction in the reproductive potential of the source population. Pollen supplementation would be especially appropriate for the populations along Caribou Road (MOST001 and MOST004), since they show the lowest genetic diversity. In the case that pollen transfers are ineffective, managers could transfer seeds among populations as prescribed above. However, our genetic data suggest that the seeds could be inbred, which could lead to lower germination rates and reproductive success.

In addition to the genetic maintenance strategies described above, we encourage detailed follow-up demographic monitoring and further basic study to better understand the life history of these rare plants. To be specific, studies of the following would be especially relevant to conservation: seed bank quantification and longevity, seed dispersal, and response to natural and anthropogenic disturbance.

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SELF-FERTILIZATION AND HERBIVORY IN A RARE ALPINE PLANT IN CALIFORNIA, *CLAYTONIA MEGARHIZA* (MONTIACEAE)

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ABSTRACT

Reproduction in alpine habitats is challenging because of the short growing season, low temperatures, and high winds. This predicts alternative strategies for sexual reproduction in plants: compensatory measures such as larger floral displays and greater floral longevity to attract scarce pollinators and maintain outcrossing, or high levels of autonomous self-fertilization to assure reproduction in the absence of reliable pollinators. Here, we assessed the roles of animals (crawling insects, flying insects, and vertebrates) on the reproductive success of *Claytonia megarhiza* (A. Gray) S. Watson (alpine spring beauty). We measured fruit set and leaf herbivory while excluding animals from individual plants at a single site in Yosemite National Park, California. We found that plants were capable of setting fruit in the absence of pollinators and that, in the presence of animals, there was a 42% reduction in fruit set and a 159% increase in leaf damage. This suggests that *Claytonia megarhiza* may reproduce primarily by self-fertilization, and that herbivory may limit the reproductive success of this species near its southern range edge in California.

Key Words: ant, breeding system, mating system, outcrossing, pollination, reproductive assurance, selfing, geographic range edge.

The reproductive biology of California's alpine plant species is poorly characterized compared to other alpine regions of the world (Körner 2003), and yet is of fundamental importance for colonization, establishment, and long-term persistence of populations and species (Schemske et al. 1994). The severity of the alpine environment—short growing season, low temperatures, intense solar radiation and strong winds—makes reproduction particularly challenging (Billings and Mooney 1968). Pollinator diversity and abundance in alpine habitats is typically limited (Billings and Mooney 1968; Bingham and Orthner 1998; Sandvik et al. 1999; Arroyo et al. 2006), and herbivore pressures can be intense (e.g., Spira and Pollak 1986; Galen 1990, 1999). Furthermore, because many of California's alpine plant species are rare (California Native Plant Society, Rare Plant Program 2016), and because alpine habitat is predicted to decrease by 50–90% across California by 2100 (Hayhoe et al. 2004), there is an urgent need to improve our understanding of the reproductive biology of California's alpine species.

Low pollinator diversity and abundance in alpine habitats favor alternative, but not mutually exclusive, pollination strategies. Plant species may evolve to have large, synchronous floral displays and increased floral longevity, which may improve the odds of successful pollination in pollinator-limited environments (e.g., Bingham and Orthner 1998; Blionis et al. 2001). In addition, alpine plants may rely on animals that are typically inefficient pollinators but are abundant in alpine habitats, such as flies and even ants (e.g., Stanton and Galen 1989; Puterbaugh 1998). Conversely, uniparental reproduction may be favored in the alpine (e.g., Spira and Pollak 1986; Gómez 2002; Zhang and Li 2008), by increasing reproductive success in the absence of reliable pollinators. This may be achieved through fertilization of ovules with self pollen (self-fertilization), or through asexual reproduction (e.g., apomixis). Over time, pollinator-limited populations may evolve to be mixed mating, predominantly self-fertilizing, or asexual, allowing for rapid reproduction and reduced investment in traits associated with pollinator attraction (Sicard and Lenhard 2011). Yet, despite the potential benefits of uniparental reproduction, it also incurs costs, such as reduced genetic variation and

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FIG. 1. *Claytonia megarhiza* is distributed in mountainous regions of western North America (left panel: white filled circles represent locations of voucher specimens, grey filled diamond indicates the study site in Yosemite National Park). Growth habit (upper and lower right) with nectaring *Formica* ant (lower right inset) near the study site in Yosemite National Park.

the accumulation of harmful mutations (reviewed in Wright et al., 2013). These costs may negatively impact a population's ability to adapt to changing environments and limit long-term population persistence (Stebbins 1957; Igic and Busch 2013). Thus, conservation strategies will differ depending on the extent to which a population or species depends on uniparental reproduction.

In addition to pollination, herbivory is another way in which animals impact plant reproduction in alpine habitats. For example, herbivory by marmots was found to limit sexual reproduction in *Gentiana newberryi* A. Gray in the White Mountains of California, and is hypothesized to favor delayed flowering until after marmots have initiated hibernation in late summer (e.g., Spira and Pollak 1986). Similarly, Galen (1990, 1999) found that phloem-feeding insects, ungulates, and ants can all limit reproduction in *Polemonium viscosum* Nutt. (alpine skypilot) in the Rocky Mountains of North America. In particular, pollinating bumblebees and flower-destroying ants exerted opposing effects on reproduction (Galen and Geib 2007). It is thus important to consider both potential pollinators and herbivores

when assessing the reproductive success of alpine plants.

Here, we assess the impact of potential pollinators and herbivores on the reproduction of an alpine plant in California – *Claytonia megarhiza* (A. Gray) S. Watson. *Claytonia megarhiza* is a perennial species that is widely distributed throughout the Rocky Mountains and the Cascades with disjunct populations in the Canadian arctic and New Mexico (Fig. 1). In California, it reaches its southern extent in the central Sierra Nevada where there are 14 documented occurrences inside Yosemite National Park. It has broad, fleshy, basal leaves and a long, stout taproot, with reports of taproots more than two meters long (Zwinger and Willard 1996). Plants only reproduce via flowers, with no vegetative reproduction. Flowers are 12–20 mm in diameter with white, pink, or rose petals (Baldwin et al. 2012). *Claytonia megarhiza* occurs in rock crevices and on loose talus, scree, or gravelly slopes, primarily in alpine habitats above treeline (Miller and Chambers 2006). In the Yosemite region, it is restricted to north facing slopes that hold snow accumulation late into summer and may thus be impacted by diminished snowpack predicted

under most climate change scenarios (Hayhoe et al. 2004). *Claytonia megarhiza* is listed by the California Native Plant Society as rare, threatened or endangered in California, but is common in other portions of its range (Rank 2B.3 in California Native Plant Society, Rare Plant Program 2016).

The reproductive biology of *Claytonia megarhiza* is poorly understood. Close relatives vary in their dependence on pollinators. *Claytonia virginica* L. relies heavily on pollinator visitation for successful reproduction [although there are reports of self-compatibility and self-fertilization of fruits at very low levels (Schemske 1977; Morgan 1998)], while *C. perfoliata* undergoes autonomous self-fertilization (Miller and Chambers 2006). There are no known reports of floral visitors to *C. megarhiza*, but ants of the genus *Formica* have been observed on flowers in Yosemite National Park (Fig. 1, Grossenbacher, pers. obs.). Because ants are generally inefficient pollinators and sometimes act as herbivores (Galen and Geib 2007), it is unclear whether they provide a net benefit or detriment to reproduction in *C. megarhiza*.

Here, we experimentally assessed the impact of animals (ants and other crawling insects, flying insects, and vertebrates) on successful reproduction of a single population in Yosemite National Park. We addressed three primary study questions: 1) Is *Claytonia megarhiza* capable of autonomous self-fertilization?, 2) Do animals positively or negatively impact fruit set and leaf damage?, and 3) In particular, do ants and other crawling insects positively or negatively impact fruit set and leaf damage, i.e., do they act as pollinators, herbivores, or both?

METHODS

Study Design

This study took place in Yosemite National Park in a *C. megarhiza* population near Ireland Lake in Tuolumne Co., CA above tree line on a NW facing granitic slope (ca. 3450–3480 m elevation, 37.775661 latitude, –119.292286 longitude). On 26 July 2008, we established three 30 m transects, each approximately 15 m apart running NE to SW (i.e., perpendicular to the NW facing slope). The three transects act as blocking factors to sample the naturally occurring elevational gradient in the experimental site (roughly 30 m elevational change). Along each transect, we identified 25 focal plants by choosing the first 25 plants that were at least 5 cm in diameter and within 0.5 m of the transect tape. We then randomly assigned five focal plants along each transect to each of the following five treatments ($n = 5$ plants/treatment/transect):

No treatment. Plants were marked with a small removable metal tag but received no treatment. This

allowed us to assess natural levels of fruit-set and herbivory.

All animals excluded. Plants were enclosed in a clear plastic flashing collar covered with netting (0.5 mm hole diameter). This mesh size effectively excludes most insects and all vertebrates, such as yellow-bellied marmots which were common at this site (Grossenbacher personal observation). We also applied the product Tree Tanglefoot (hereafter “Tanglefoot”, The Tanglefoot Company, Grand Rapids, MI) to further prevent access by crawling insects. Tanglefoot is a commercial product that provides a sticky surface that crawling insects cannot cross. This treatment tested whether excluding all potential pollinators and herbivores had an effect on fruit-set and herbivory, and specifically tested for autonomous self-fertilization.

Crawling insects excluded. Plants were enclosed in a plastic flashing collar and Tanglefoot was applied to impede access by ants and other crawling insects. Flying insects and vertebrates still had access to the plants. This treatment tested whether excluding crawling insects had an effect on fruit-set and herbivory.

Control A. To evaluate the effect of plastic flashing, plants were enclosed in a plastic flashing collar, but no Tanglefoot or netting was applied. This allowed all potential pollinators and herbivores to access plants, and tested whether flashing had an effect on fruit-set and herbivory. For example, the roughly 4 cm tall flashing may act as a wind barrier, influencing air flow and temperature around plants.

Control B. To evaluate the effect of netting, plants were enclosed in a plastic flashing collar covered with netting, with holes cut in the netting. This allowed all potential pollinators and herbivores to access the plants and tested whether the shade created by netting had an effect on fruit-set and herbivory.

Treatments were implemented on 26 July 2008 immediately after snowmelt. When flowering was complete for nearly all plants (i.e., there were no new developing flowers or inflorescences), we censused the population (9 September 2008). We assessed reproductive success by counting the number of set fruits per plant, which equaled the sum of undehisced and dehisced capsules. To estimate plant size and herbivore damage, we counted the total leaf number per plant, and estimated the amount of herbivore damage by visually surveying leaves for damage. A leaf was categorized as herbivorized if greater than 25% of the surface was missing or damaged (evidence of insect sucking or chewing scars or browsing). We used the remaining leaf margins to estimate the original leaf size and therefore the percent damage. For each individual plant, the percent leaf damage was equal to the number of damaged leaves divided by the total leaf number per plant.

TABLE 1. FRUIT SET AND PERCENT LEAF DAMAGE AS RESPONSE VARIABLES IN TWO SEPARATE GENERALIZED LINEAR MODELS (A), AND THEIR ASSOCIATED PRE-PLANNED CONTRASTS (B). “F” indicates F value, “z” indicates Z value, and subscript “df” indicates the degrees of freedom.

(A) Overall model	Fruit set		Percent leaf damage	
	F _{df}	P	F _{df}	P
Source of variation				
Exclusion treatment (5 levels)	2.22 ₄	0.078	3.75 ₄	0.008
Transect (3 levels)	2.86 ₂	0.065	4.73 ₂	0.012
Exclusion treatment X transect	0.49 ₈	0.857	2.48 ₈	0.022
(B) Pre-planned contrasts	Z _{df}	P	Z _{df}	P
Source of variation				
All animals excluded versus all other treatments	2.71 ₁	0.007	−3.61 ₁	<0.001
Crawling insects excluded versus No treatment, Control A, and Control B	1.16 ₁	0.245	−0.39 ₁	0.696
Control A versus No treatment	0.31 ₁	0.758	−0.55 ₁	0.586
Control B versus No treatment and Control A	−0.36 ₁	0.716	−1.61 ₁	0.107

Statistical Analyses

To examine whether individual fruit set and percent leaf damage varied by exclusion treatment (five treatments), transect (three transects), and the interaction of treatment and transect, we used two separate generalized linear models (GLM) in the *MASS* package in R (Venables and Ripley 2002). Both treatment and transect were treated as fixed factors. We considered transect a fixed factor because there were only three transects and we chose them deliberately with respect to elevation. A negative binomial error distribution was used for both models because the response variables (number of set fruits and percent leaf damage) were overdispersed, and because this distribution provided the best fit to the data error structure and allowed for the use of pre-planned contrasts. In both models, we included pre-planned contrasts to specifically address study question 1 and 2 (contrast: *All animals excluded* versus all other treatments) and study question 3 (contrast: *Crawling insects excluded* versus *No treatment, Control A*, and *Control B*). Two additional preplanned contrasts were used to examine whether there was an effect of plastic flashing (contrast: *Control A* versus *no treatment*) or netting (contrast: *Control B* versus *No treatment* and *Control A*). Code for all analyses and raw data files are available from the Dryad Digital Repository (doi:10.5061/dryad.tr20q).

RESULTS

Fruit set marginally varied by exclusion treatment ($P = 0.078$) and transect ($P = 0.065$), but not the interaction of treatment and transect ($P = 0.857$) in a generalized linear model (Table 1A, Fig. 2A). Pre-planned contrasts demonstrated that animals caused a 42% reduction in fruit set on average compared to when all animals were excluded ($P = 0.007$; Table 1B). All other planned contrasts did not reveal significant differences among treatments, i.e., there

was no effect of crawling insects, plastic flashing or netting on fruit set (Table 1B). Leaf damage significantly varied by exclusion treatment ($P = 0.008$), transect ($P = 0.012$), and by the interaction of treatment and transect ($P = 0.022$) in a generalized linear model (Table 1A, Fig. 2B). Pre-planned contrasts demonstrated that animals caused a 159% increase in leaf damage compared to when all animals were excluded ($P < 0.001$; Table 1B). All other contrasts were not significant, i.e., there was no effect of crawling insects, plastic flashing or netting on leaf damage (Table 1B).

DISCUSSION

We demonstrated that *Claytonia megarhiza* undergoes autonomous self-fertilization in the absence of pollinators at a single site in the central Sierra Nevada. Overall, reproduction appears to be negatively impacted by animals: there was a 42% reduction in fruit set and a 159% increase in leaf herbivory in the presence of animals. We found no evidence that ants or other crawling insects acted as either pollinators or herbivores, despite the prior observation that ants are floral visitors of this species in Yosemite National Park (Fig. 1). Our results suggest that *C. megarhiza* may be predominantly self-fertilizing near its southern range edge in California. The evolution of self-fertilization is predicted to be greater in range edge and peripheral populations, which may suffer from marginal environmental conditions (Busch 2005; Herlihy and Eckert 2005; Moeller et al. 2012). For example, reduced or unfavorable growing seasons may necessitate shorter reproductive cycles, which favor self-fertilization or mixed mating to provide for reproductive assurance. Range edge populations may also experience greater mate- or pollinator-limitation, which also favor self-fertilization (Moeller and Geber, 2005; Moeller 2006; Moeller et al. 2012). For *C. megarhiza*, an alpine plant already subject to harsh environmental conditions, the range edge

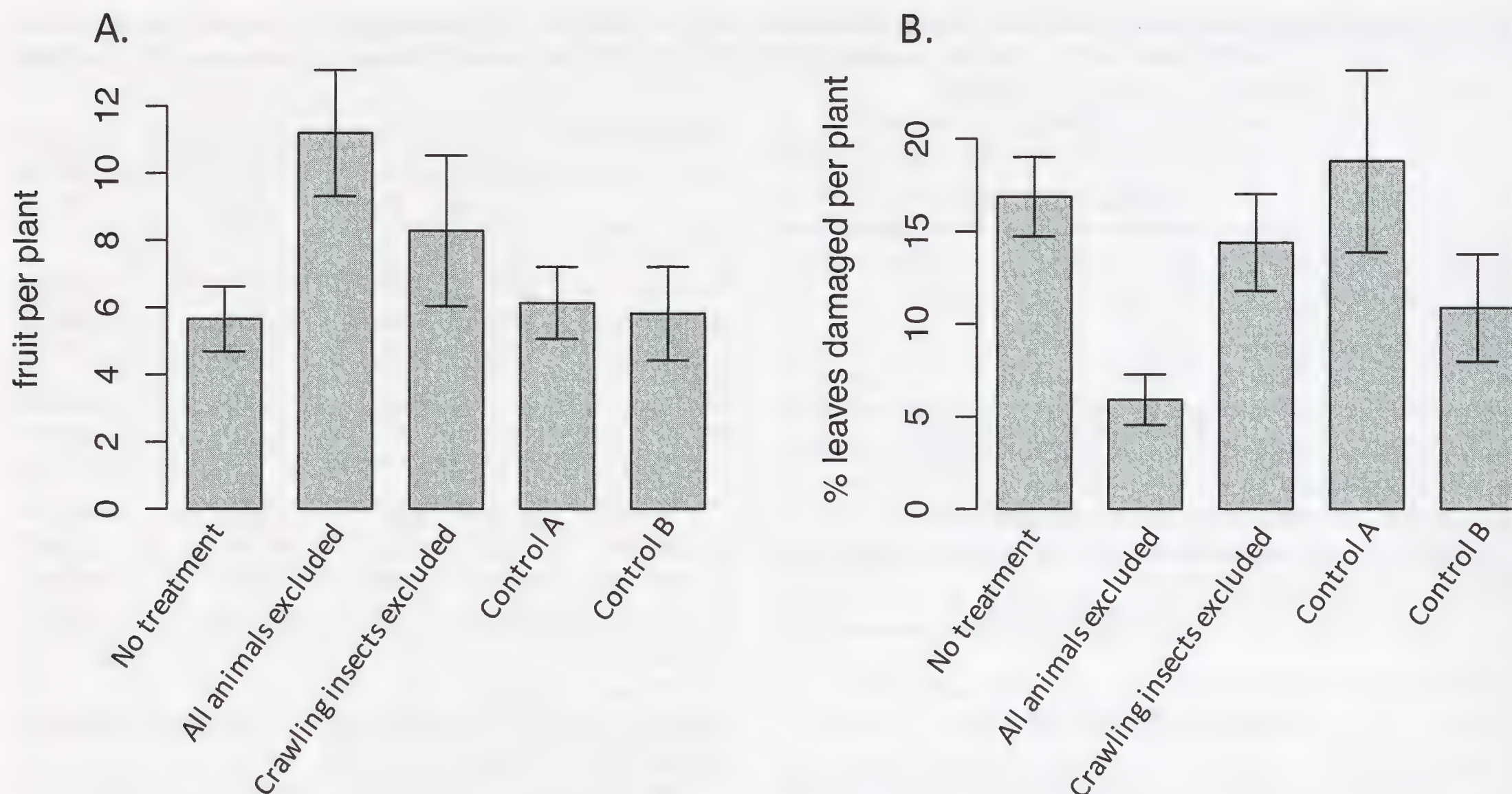


FIG. 2. Observed mean fruit set (A) and mean percent leaf damage (B) per exclusion treatment \pm 1SE. Experimental treatments included: “No treatment” where plants were left unmanipulated; “All animals excluded” where netting, plastic flashing, and ‘Tree Tanglefoot’ impeded access by most insects and vertebrates; “Crawling insects excluded” where plastic flashing and ‘Tree Tanglefoot’ impeded access by ants and other crawling insects; “Control A” where plastic flashing without ‘Tree Tanglefoot’ allowed all animals to access plants; “Control B” where plastic flashing was covered with netting with holes to allow animals to access plants.

position may further challenge reproductive success and lead to the evolution of greater self-fertilization. Furthermore, *C. megarhiza* is reported to vary in ploidy among populations ($2n = 12, 16, 24, 32, 34, 36$; Baldwin et al. 2012), which could influence variation in breeding system as polyploidy is often associated with shifts to self-fertilization (Barringer 2007). Careful investigation of *C. megarhiza* in core and range edge habitats will help to better understand how these environments impact reproduction.

Consistent with increased self-fertilization at the study site, there appears to be reduced investment in pollinator attraction traits. We noted that petals of opened flowers at our study site were just 3–4 mm long, and are among the smallest reported for this species (e.g., a range of 5–9 mm is reported in Baldwin et al., 2012). Additionally, the petals were paler in color compared with those found in some northern populations (Grossenbacher, pers. obs.). A trend toward reduced floral investment following the evolution of predominant selfing has been found in many species (i.e., Sicard and Lenhard 2011). Nonetheless, some outcrossing may occur in this focal population, as our experimental design did not rule out potential for a mixed-mating strategy. For example, some cross-pollination may have occurred in treatments that permitted floral visitors, while delayed autonomous self-fertilization provided a backup mechanism in treatments where visitors were excluded. Determining the extent of cross-pollination near the southern range edge will require genetic data

to estimate rates of self-fertilization in untreated plants.

Knowledge of the reproduction of *C. megarhiza*, a rare species in California, can be used for management in several ways. When establishing new populations or restoring existing populations, the ability of this species to produce self-fertilized seed might allow for the successful establishment of relatively few individuals, as they will effectively produce seed without cross-pollination. Given the high rates of leaf herbivory and reduced fruit set in the presence of animals, temporary animal exclosures may help new plants to establish and boost reproductive output of existing plants. Finally, existing populations could be significantly differentiated from one another due to the rapid accumulation of genetic changes in predominantly selfing lineages and may exhibit high levels of fixed genetic load due to the accumulation of harmful mutations (reviewed in Wright et al., 2013). This may need to be taken into account if seed is to be transferred among populations or used to establish new populations.

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ESTIMATING BIOMASS IN CALIFORNIA'S CHAPARRAL AND COASTAL SAGE SCRUB SHRUBLANDS

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ABSTRACT

Despite being one of the most extensive vegetation types in the North American Mediterranean Climate Zone, information on the amount of biomass and carbon stock associated with shrubland vegetation is still largely unknown. Efforts to quantify shrubland biomass through fieldwork consist of either direct measurement using destructive vegetation sampling or indirect measurement through the combined use of stem measurements and allometric equations. Both methods have their own benefits and shortcomings, resulting in substantial variation in how shrubland biomass is reported. Here we aim to provide a comprehensive review and synthesis of available shrubland biomass data from studies based in California to provide a concise and reliable source for natural resource managers. We conducted a literature review of 37 studies published over a span of 72 yrs to compile estimates of aboveground biomass (live, dead, and total), leaf biomass, stem biomass (live, dead, and total), litter biomass, and belowground biomass for three prominent shrubland communities and four shrubland species in the California Floristic Province. Overall aboveground biomass in shrub communities was greatest in mixed chaparral (3461 g/m²), followed by chamise chaparral (2114 g/m²), and coastal sage scrub (1583 g/m²). In each community total aboveground biomass increased with the age of the stand. Leaf, stem, and litter biomass estimates were also highest for mixed chaparral compared to the other communities. Of the four shrub species we summarized biomass data for, *Ceanothus greggii* A. Gray (Rhamnaceae) had the highest average aboveground biomass, followed by *Adenostoma fasciculatum* Hook. & Arn. (Rosaceae), *Quercus berberidifolia* Liebm. (Fagaceae), and *C. cuneatus* (Hook.) Nutt. By compiling these studies and summarizing the biomass data reported in them, we provide a single resource to characterize the amount of biomass of three different shrublands and four species over their life cycle. This is an essential resource for land managers and practitioners who need field-based biomass and carbon stock figures for monitoring and reporting purposes.

Key Words: Aboveground biomass, *Adenostoma fasciculatum* Hook. & Arn., belowground biomass, California, carbon storage, *Ceanothus* sp., chaparral, coastal sage scrub, North American Mediterranean Climate Zone, *Quercus berberidifolia* Liebm.

Shrublands cover extensive areas of the North American Mediterranean Climate Zone (also known as the California Floristic Province), which includes most of California, southwestern Oregon, northwestern Baja California, Mexico, and a sliver of western Nevada. However, despite their large spatial extent there is little appreciation of the contribution shrublands make to carbon storage and carbon sequestration (Di Castri et al. 1981, Luo et al. 2007). Chaparral and coastal sage scrub are two highly characteristic shrubland types of the lowland and middle elevation of this Mediterranean Climate Zone, especially in the southern portions of California and northwest Baja California. These ecosystems support high levels of plant species richness and

endemism (Burge et al. 2016), as do their analogues in other Mediterranean-type ecosystems globally (Cowling et al. 1996, Myers et al. 2000). However, very little is known of their contributions to carbon storage or cycling other than the fact that many species have extensive and deep root systems that can provide substantial belowground carbon storage (Kummerow et al. 1977, Luo et al. 2007).

Studies and data for estimating biomass and carbon storage for major habitat types dominated by forest, such as tropical rainforests, are routinely conducted and reported. For example, some early studies providing image-derived biomass estimates for tropical rainforests were instrumental in highlighting their critical role in the global carbon cycle

(e.g., Foody et al. 2001, Saatchi et al. 2007, Propastin 2013). Within the North American Mediterranean Climate Zone, studies estimating biomass for forested portions of the landscape have been conducted, for example, for the Sierra Nevada of California (McGinnis et al. 2010, Zhang et al. 2014) and forests of northern California and southwestern Oregon (Hudiburg et al. 2009). However, studies estimating biomass of shrublands in Mediterranean climates, either in North America or in the four other regions of the world where this climate occurs, are comparatively rare (e.g., Cerrillo and Oyonarte 2006).

At the continental scale in the USA, shrubland mapping efforts generally underestimate the amount of biomass in shrublands. National scale datasets such as the National Biomass and Carbon Dataset (<http://www.whrc.org/mapping/nbcd/>) and Land-Carbon (<http://landcarbon.org/>) use USDA Forest Service Forest Inventory and Analysis (FIA) plot data alongside Landsat imagery and occasionally radar remote sensing imagery. The FIA database includes shrubland plots but unlike in forest plots, aboveground biomass is not measured in shrub plots, thus carbon mapping relying on the FIA dataset shows shrubland carbon storage as zero. At the California state scale, efforts to map carbon include the California Department of Forestry and Fire Protection's biomass assessment of forests and shrublands (FRAP 2005). Estimates are based on integrating data from land cover datasets, fuel models, as well as other spatial data on slope, land cover, ownership and fire threat (Anderson 1982, FRAP 2002, 2003, 2005).

Alternatively, the California Forest and Rangeland Greenhouse Gas Inventory assessment (Battles et al. 2014) used USGS Landfire data (30m) on Existing Vegetation Type (EVT) and the associated information on vegetation height and cover, and generated biomass densities by shrub-height classes based on the literature. Note that none of the mapping efforts described above address biomass stored belowground, which can be substantial, accounting for up to 41% of the species biomass in *Adenostoma fasciculatum* Hook. & Arn. (Kummerow et al. 1977) and 47% in *Arctostaphylos glauca* Lindl. (Miller and Ng 1977). At finer spatial scales, studies reporting field measurements of biomass in shrublands are characterized by a lack of consistency in the data collection methods and reporting. These inconsistencies are largely due to the variation in the focus of different studies and the research questions they aim to answer. Field studies vary in plot and sample size and biomass is reported using a variety of different metrics making extrapolations over larger shrubland areas challenging, given the variation in elevation (300–3000 m), soils, topography, aspect, coastal or desert exposure, and disturbance history.

Site specific influences on biomass make generalizations over large landscapes difficult. Plant community composition and structure are largely a direct reflection of the environment. At the regional scale,

precipitation drives the distribution of mixed chaparral, chamise, and sage scrub, with mixed chaparral found in comparably wetter areas than both chamise and sage scrub (Conrad et al. 1986). In more productive sites, for example, with higher annual precipitation, higher annual production drives higher biomass accumulation (Gray 1982, Keeley and Keeley 1988, Schlesinger and Gill 1980, Uyeda et al. 2016). At a local scale, these differences in productivity can be due to slope, aspect, elevation, and soil characteristics (Hellmers 1955, Keeley and Keeley 1988, Regelbrugge and Conard 1996, Riggan and Dunn 1982, Schlesinger et al. 1982).

One reason for the lack of comprehensive data on shrubland biomass is the physical difficulty of conducting fieldwork in these systems. Dense stands of shrublands, particularly chaparral, are often described as 'impenetrable' and slopes are often steep, consequently undertaking systematic fieldwork requires substantial resources and effort (Uresk and Menke 1977). Executing full shrub harvests to directly measure above- and belowground biomass requires excessive time and resources and is not feasible to do at a large scale. A more common field method for assessing biomass combines destructive and non-destructive sampling. First, shrubs representative of the stand are selected and all aboveground biomass is collected. Plant material is then dried and weighed, occasionally being broken down into different stem size classes, plant parts and/or live and dead material. These shrub samples are then used to build species-specific regression equations that relate more easily measured variables (e.g., stem diameter, cover) to overall biomass. Ideally the roots are also excavated, however in most cases belowground biomass is either ignored or calculated based on pre-determined root:shoot ratios (Mooney and Rundel 1979).

The variation in the locations and site specific characteristics of field studies combined with the lack of accurate image-derived maps at a useful scale, create a need for the available biomass data to be synthesized in a concise and comprehensive way. From a resource management perspective, there is an increasing need to be able to accurately quantify biomass in shrubland landscapes and understand how it is impacted by management actions or by disturbance events such as wildfire. Similarly, information on the projected recovery of biomass post disturbance allows resource managers to anticipate the return of carbon storage ecosystem services and also the return of other services associated with vegetation and biomass, such as wildlife habitat or an established root system for retaining sediment, which is otherwise eroded from denuded slopes (Wohlge-muth et al. 1999). If disturbances become too frequent in shrubland communities (e.g., extremely short fire-return intervals) they can result in vegetation type-conversions, changing the nature of carbon storage and sequestration on the landscape as biomass values differ substantially between shrub-

lands and non-native annual grasses (Keeley et al. 2005, Lippitt et al. 2013).

From a policy perspective, federal agencies such as the USDA Forest Service are now required to record and report information on carbon stocks. The Climate Change Scorecard for example, requires the quantity of carbon stocks under Forest Service management to be reported as well as the estimated impact of disturbance and management activities on these carbon stocks (<http://www.fs.fed.us/climatechange/advisor/scorecard/carbon-assessment-stewardship.html>). There is also increasing interest in mapping and quantifying the ecosystem services that natural landscapes provide, which can – among other things – provide the basis for developing market based approaches such as Payment for Ecosystem Services (Chan et al. 2006). In this paper we review and summarize the available literature on above- and belowground biomass of shrublands from studies based in California with the intention of providing a concise and reliable resource for natural resource managers.

METHODS

To compile the available data on biomass we carried out an extensive literature search online. We used the Web of Science and Google Scholar search engines specifying key words that included ‘chaparral’, ‘shrubland’, ‘biomass’, ‘chamise’, ‘sage scrub’, and ‘California’. In addition, more general web searches were conducted to locate government documents or conference and symposium proceedings. From the relevant literature we extracted all reported biomass values and associated details including location (general and specific coordinates), sample size, parent material, elevation, aspect, age, stand height, unit of measurements, and species mix (when specified). In some cases biomass values were presented in graphical form rather than tables in which case it was necessary to approximate the actual values. We also recorded specific details of the biomass measurements, for example, whether reported values were calculated using destructive sampling, allometric equations, and/or whether dead material was included in the total biomass values. Studies that reported biomass values in a format that did not allow for comparisons with other studies were excluded (e.g., biomass per plant versus biomass per area).

To synthesize the array of different datasets we made the following assumptions. When only certain biomass components were reported we calculated the missing components when feasible. For example, if live and dead biomass values were reported without reporting a total aboveground biomass, we summed live and dead to report total aboveground biomass. Alternately, if a total aboveground biomass value and a live aboveground biomass value were reported without a value for dead biomass, we subtracted live from the total to provide a value for dead biomass.

Finally, if aboveground biomass was reported without specifying live or dead, we assumed both were included. This last assumption may lead to an underestimate of total aboveground biomass, with combined live and dead averages occasionally exceeding total aboveground averages. Similarly for species-level data, when only live aboveground biomass was reported, we included it when averaging total aboveground biomass which may cause minor underestimates of species-level total aboveground biomass due to the missing dead biomass component. Consequently, in using these data we caution that some interpretation was necessary where the published material was unclear.

Once collected, we summarized the studies based on community type, age of shrubland and species. Community type was determined based on either the designation provided by the authors of the given study or assigned based on the shrub species reported for the given stand. Mixed chaparral stands varied in the number of species present and at times were largely dominated by one or two chaparral species such as *Ceanothus greggii* A. Gray or *Quercus berberidifolia* Liebm. Only pure stands of *Adenostoma fasciculatum* or stands that were explicitly dominated by *A. fasciculatum* were classified as chamise chaparral. In some cases, biomass averages of monospecific shrub communities were included in the species breakdown. It was assumed that the stand level biomass per unit area was the same as the shrub level biomass per unit area when there was only one species present in the stand. For example, a pure stand of *A. fasciculatum* included in the chamise chaparral summaries, was also included in the species summary for *A. fasciculatum*.

We summarized data for shrub communities and species that were sufficiently documented in the literature. Shrub communities with less than three studies reporting overall biomass, such as desert shrubland, are not reported. Data were grouped into different age classes (e.g., 1–10, 11–20, 21–30, >30 yr) and summarized accordingly. When shrubland age was not provided, effort was made to determine the general age of the stand through descriptions in the text. For instance, if a stand was described as “mature” but not assigned a specific age, it was placed in the oldest age class. If no age was provided nor was there sufficient information to assign an age class, the associated data were included in the overall averages but not in a specific age class. It is important to note that in most studies, the age of shrubland was determined by the time of last fire. Furthermore, biomass metrics reported in different studies varied widely with little consistency in the units of measurement used, which included tons/acre, lbs/acre, g/m², kg/m², Mg/ha, kg/ha, and tonnes/ha. We standardized all biomass units to g/m².

We report the available biomass studies at two spatial scales. First by community type: mixed chaparral, chamise chaparral, and coastal sage scrub; and second, at a higher resolution using four

dominant shrubland species: *Adenostoma fasciculatum*, *Ceanothus greggii*, *Quercus berberidifolia*, and *C. cuneatus*. Subdividing the data by stand age, we summarize total aboveground biomass and range and the live and dead aboveground biomass and range (for the community level only). These were the most commonly reported variables in the studies reviewed. Other data we present include annual aboveground biomass increment, a breakdown of live and dead stem biomass, leaf biomass, litter biomass, and root-to-shoot ratios.

Finally, as a case study, we examined the relationship between aboveground biomass and one of the key drivers in productivity – precipitation. We mapped a subset of study locations ($n = 36$) and display these with annual mean precipitation from the Basin Characterization Model (BCM) dataset (1981–2010, Flint et al. 2013). Where a single location had multiple biomass measurements for different age classes of chaparral, we averaged measurements of stands >10 yr old (this allowed us to keep most of the plots except for the very young ones). For these 36 plots we identified mean annual precipitation values and assessed the correlation with mean aboveground biomass.

RESULTS

We reviewed 37 studies published between 1944 and 2016 with the majority published in the 1970s and 1980s. While we did our best to capture as much of the relevant literature as possible using the keyword searches described, it is inevitable that some studies might have been missed owing to time and resource constraints. Just over half of these documents (51%) were published in peer-reviewed journals and sources for the remaining documents included conferences and/or symposium proceedings (14%), government documents (14%), dissertations (11%), book chapters (8%), and university reports (3%). The most common shrub community types reported in the literature were mixed chaparral and chamise chaparral, followed by coastal sage scrub and desert shrublands. Many studies also reported biomass at the species level, with 75% of these studies reporting biomass values for *A. fasciculatum*. Other species such as *Q. berberidifolia*, *C. greggii*, and *C. cuneatus* were less prevalent in the literature, each reported in 15% of the studies. There were 21 additional species reported on but much less frequently.

Of the biomass metrics reported in the 37 studies, total aboveground biomass was reported most frequently (almost 90% of the studies), followed by live aboveground biomass, and dead aboveground biomass. Litter biomass, leaf biomass, live and dead stem biomass, and aboveground annual increment of biomass (i.e., accumulated biomass per yr) were less commonly reported ($< 33\%$ of the studies). Belowground biomass was the least common biomass metric, reported in only 16% of the studies.

Aboveground Biomass Estimates

Biomass at the community level. Mixed chaparral was the most widely reported shrub community in the literature. Summaries show that average total, live, and dead aboveground biomass were all lowest in 1–10 yr old stands (Table 1, Fig. 1). In these young stands total aboveground biomass on average was 861 g/m^2 with an average of 792 g/m^2 of live aboveground biomass and 300 g/m^2 of dead aboveground biomass. Total and dead aboveground biomass was the highest on average in stands >30 yr old with 4931 g/m^2 and 1575 g/m^2 respectively. Live aboveground biomass was the highest on average in 21–30 yr old stands with 4674 g/m^2 (Table 1, Fig. 1). The highest aboveground biomass reported among studies focusing on mixed chaparral was $11,800 \text{ g/m}^2$ in a 55 yr old *Ceanothus*-dominated stand (Regelbrugge and Conard 1996). Average annual aboveground biomass increment was highest in 21–30 year old stands at 952 g/m^2 , however annual increment data reported for older stands (>30 yr old) were limited to only one study (Riggan and Lopez 1982; average biomass increment 67 g/m^2), so this value is unlikely to be representative of older mixed chaparral stands as a whole.

Chamise chaparral was the second most commonly reported shrub community in the literature (Table 2). There were no studies that report comparable biomass values for chamise chaparral in the 21–30 yr age class. Due to the lack of data for this age class, it was necessary to combine the 11–20 and 21–30 age classes. The lowest values for average aboveground total, live and dead biomass were found in the youngest stands (1–10 yr; Fig 2). Average total aboveground biomass in this age class was very similar to that of the young mixed chaparral stands with a value of 923 g/m^2 . On average, total, live and dead aboveground biomass were all highest in >30 yr old stands. The highest reported total aboveground biomass was 4909 g/m^2 in a 37 yr old *A. fasciculatum* stand with a small component of *Ceanothus crassifolius* Torr. present (Specht 1969). Average biomass in >30 yr old chamise chaparral stands was about half that reported in equivalent stands of mixed chaparral (2787 g/m^2 and 4931 g/m^2 respectively).

In contrast, biomass data from coastal sage scrub stands were relatively limited and data only supported a breakdown into two age classes, 1–10 yr and >10 yr (Table 3). Average total and live aboveground biomass were both lowest in 1–10 yr old stands with 598 g/m^2 and 409 g/m^2 respectively (Fig. 3). Dead aboveground biomass for this age group was not reported in the literature. Average total and live aboveground biomass were highest in stands >10 yr old (the oldest stand reported on was 40 yr). Total aboveground biomass for older stands averaged 1901 g/m^2 , with average live of 995 g/m^2 and average dead of 555 g/m^2 .

Data on leaf, stem, and litter biomass were less commonly reported in the studies reviewed. Leaf

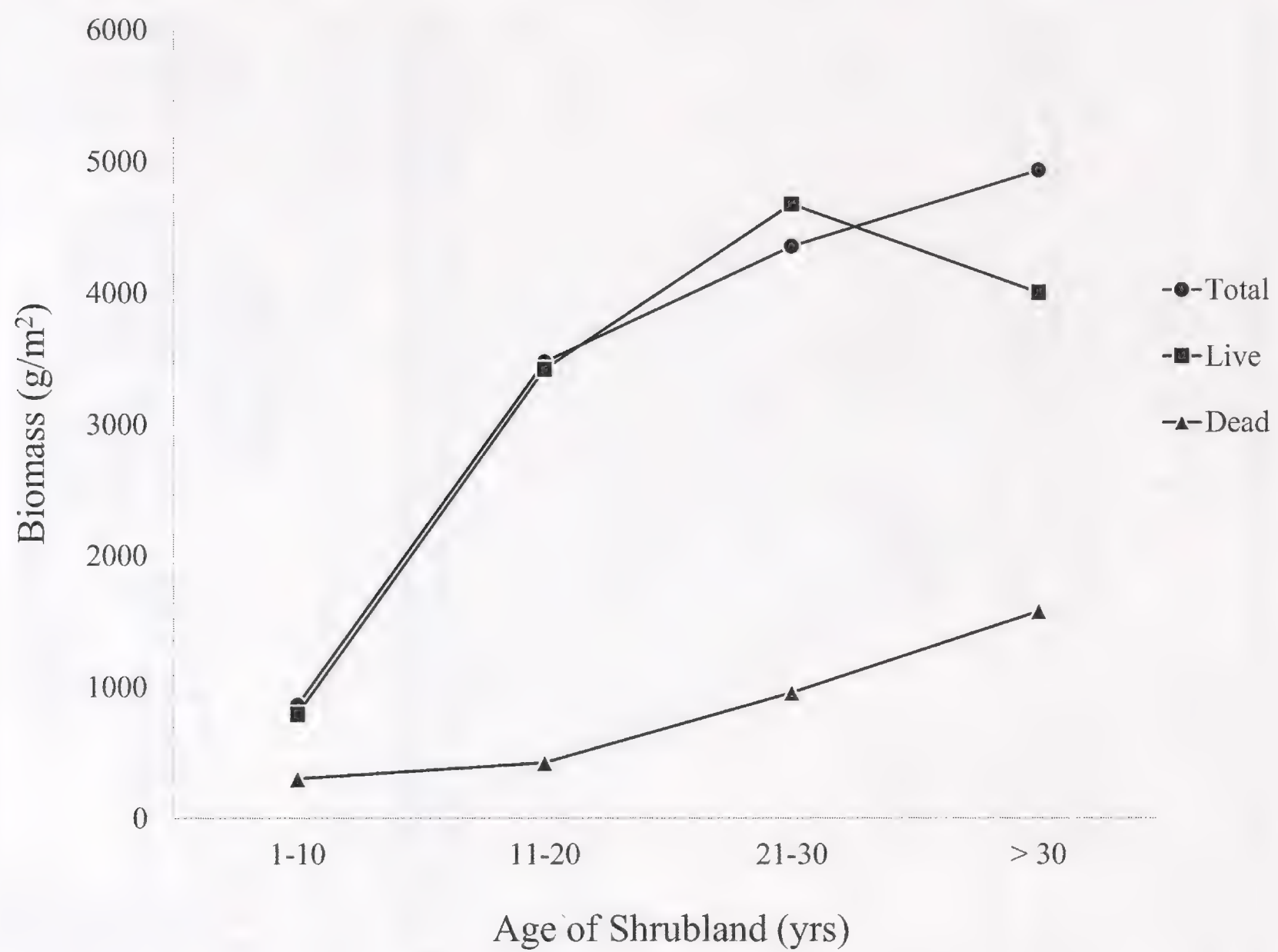


FIG. 1. Estimates of aboveground biomass (g/m^2) for mixed chaparral by age class based on 25 studies with biomass measurements from 1944–2016. Note: average total aboveground biomass does not equate to live plus dead because not all studies reported all three variables.

biomass in mixed chaparral was approximately twice that of coastal sage scrub communities (303 g/m^2 compared to 141 g/m^2 ; Tables 1 and 3). Similarly, total stem biomass was about four times greater in mixed chaparral compared to coastal sage scrub

(4199 g/m^2 compared to 1120 g/m^2). The amount of litter across all age classes was highest in mixed chaparral followed by coastal sage scrub and then chamise chaparral communities (2541 g/m^2 , 1392 g/m^2 , and 1278 g/m^2 respectively).

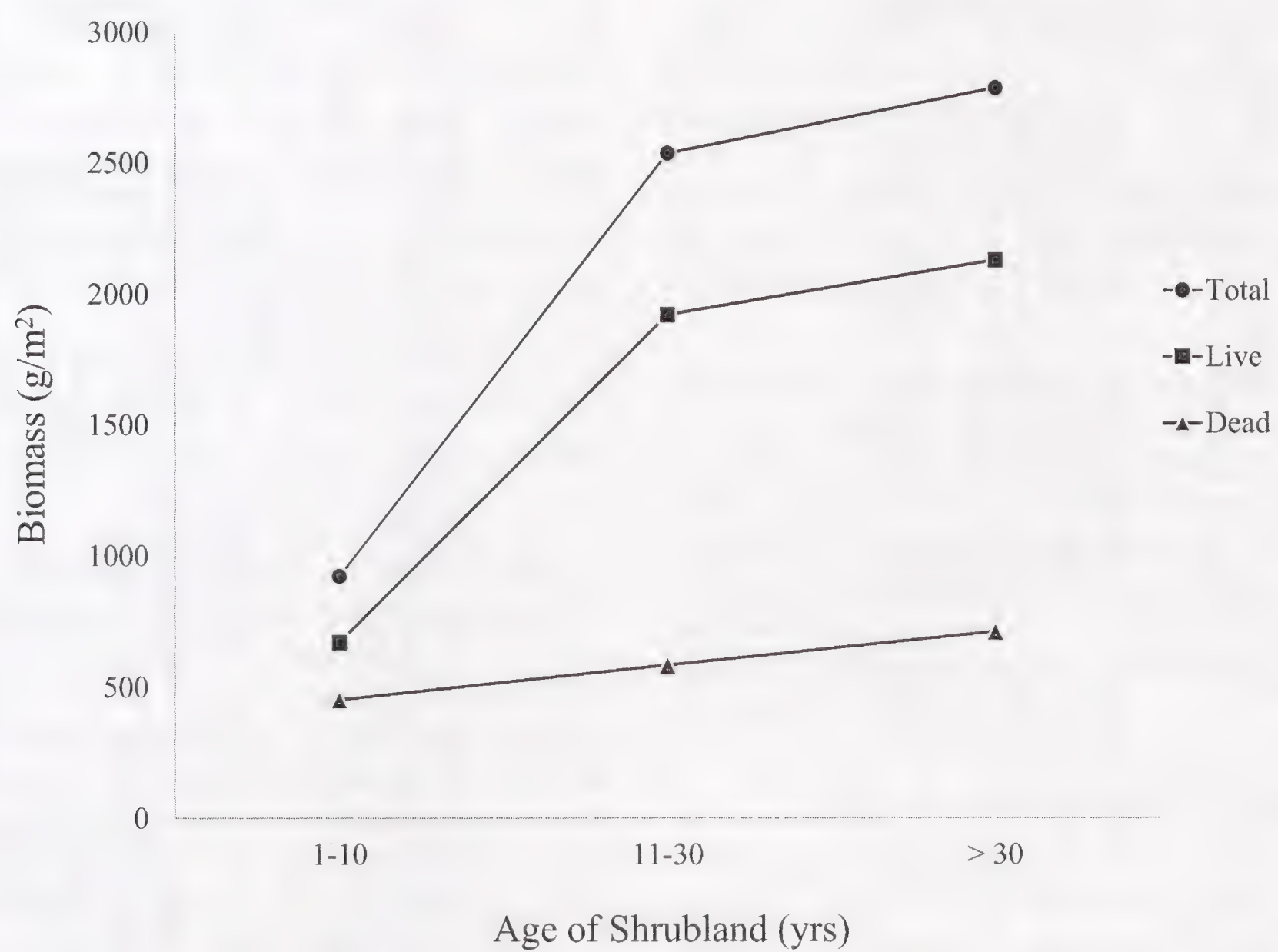


FIG. 2. Estimates of aboveground biomass (g/m^2) for chamise chaparral by age class based on 13 studies with biomass measurements from 1944–2012. Note: average total aboveground biomass does not equate to live plus dead because not all studies reported all three variables.

TABLE 3. SUMMARY OF COASTAL SAGE SCRUB BIOMASS DATA BASED ON 10 STUDIES WITH BIOMASS MEASUREMENTS FROM 1981–2012 IN THE AVAILABLE LITERATURE [REFERENCES 2, 3, 5, 7, 8, 10, 14, 21, 35, AND 37 IN APPENDIX 1]. Note: average total aboveground biomass does not equate to live plus dead because not all studies report all three variables. Total includes all age classes as well as studies that did not provide a shrubland age. ⁱValues averaged from ≤ 2 studies.

Age class (yrs.)	Total aboveground biomass (g/m ²)		Live aboveground biomass (g/m ²)		Dead aboveground biomass (g/m ²)		Avg biomass increment (g/m ² /yr.)	Avg leaf biomass (g/m ²)	Avg live stem biomass (g/m ²)	Avg dead stem biomass (g/m ²)	Avg total stem biomass (g/m ²)	Avg litter biomass (g/m ²)	Avg belowground biomass (g/m ²)
	Min-Max		Avg		Avg								
	Avg	Min-Max	Avg	Min-Max	Avg	Min-Max							
1–10	597.8 ⁱ	255–958 ⁱ	409.4 ⁱ	200–630 ⁱ	—	—	306.9 ⁱ	—	—	—	—	—	—
> 10	1901.1	1172–2800	995.0	400–1300	555.4	247–852	305.1 ⁱ	140.8 ⁱ	867.3 ⁱ	253.1 ⁱ	1120.4 ⁱ	1370.9 ⁱ	—
Total	1582.7	255–3094	660.3	200–2200	617.7	247–919	306.5	140.8 ⁱ	867.3 ⁱ	253.1 ⁱ	1120.4 ⁱ	1392.2 ⁱ	—

TABLE 4. SUMMARY OF BIOMASS MEASUREMENTS FOR 4 DOMINANT CHAPARRAL SPECIES BASED ON 20 STUDIES WITH BIOMASS MEASUREMENTS FROM 1944–2012 IN THE AVAILABLE LITERATURE [REFERENCES 4, 7, 9, 12, 13, 15, 16, 18–20, 22, 25–32, AND 35 IN APPENDIX 1]. Note: some ages inferred based on stand age or age of surrounding shrubs. ⁱValues averaged from ≤ 2 studies.

Species	Age range of shrubs included (yrs)	Total aboveground biomass (g/m ²)		Avg annual biomass increment (g/m ² /yr.)	Avg leaf biomass (g/m ²)	Avg total stem biomass (g/m ²)	Avg litter biomass (g/m ²)	Total belowground biomass (g/m ²)		Avg root:shoot ratio
		Min-Max						Avg		
		Avg	Min-Max					Avg	Min-Max	
<i>Adenostoma fasciculatum</i>	1–60+	1957.6	198–6818	252.8	246.7	1136.3	—	1632.7	90.3–4789	0.6 ⁱ
<i>Ceanothus greggii</i>	21–24	4876.8 ⁱ	1109–10139 ⁱ	595.5 ⁱ	—	1275.3 ⁱ	2217.3 ⁱ	798.4 ⁱ	154.8–1442 ⁱ	0.3 ⁱ
<i>Quercus berberidifolia</i>	23–35+	1709.5	1233–2046	238.2 ⁱ	310.0 ⁱ	1695.7 ⁱ	5300.0 ⁱ	1330.0 ⁱ	—	—
<i>Ceanothus cuneatus</i>	15–25	880.6	814–990	—	46.9 ⁱ	767.0 ⁱ	—	—	—	—

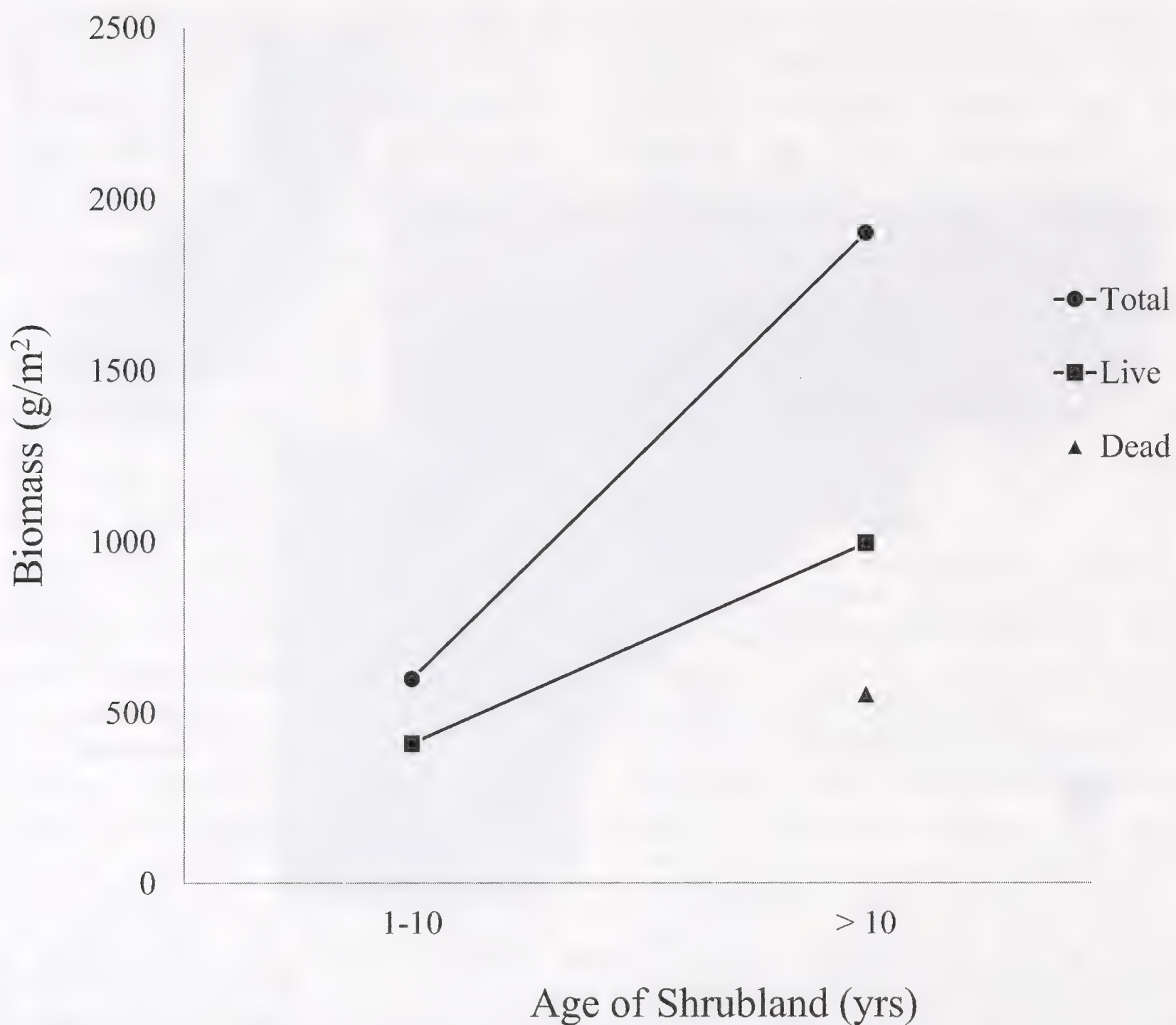


FIG. 3. Estimates of aboveground biomass (g/m^2) for coastal sage scrub based on 10 studies with biomass measurements from 1981–2016. Note: average total aboveground biomass does not equate to live plus dead because not all studies reported all three variables.

Biomass at the species level. At a finer species-level scale, we report on four dominant chaparral species based on their prevalence in the literature (Table 4). We only report total aboveground biomass, excluding a breakdown of live and dead aboveground biomass due to insufficient data. The most frequent shrub species measured was *A. fasciculatum*. Average total aboveground biomass for *A. fasciculatum* was 1958 g/m^2 (Table 4). The lowest reported biomass in the literature was 198 g/m^2 for a 1 yr old individual (Sparks and Oechel 1993) and the highest was 6818 g/m^2 reported by Kummerow et al. (1977) for a 2 yr old individual. Kummerow et al. (1977) note that this individual was resprouting and had numerous dead stems resulting in a root:shoot ratio of 0.7. The next highest biomass reported in the literature for *A. fasciculatum* was for a mature individual with a biomass of 4260 g/m^2 ($3,363 \text{ g/m}^2$ of which was live; Green 1970). Average total aboveground biomass was the highest for *C. greggii*, which also had the maximum biomass reported at $10,139 \text{ g/m}^2$ for one individual (no age provided), although 20–30% of this biomass was from dead stems (Kummerow et al. 1977). Biomass reported for *Q. berberidifolia* was lowest in a mature shrub at 1233 g/m^2 (Green 1970) and highest in a 23 yr old shrub at 2046 g/m^2 (Mooney et al. 1977). *C. cuneatus* had the lowest average total aboveground biomass as well as the smallest range of biomass values reported with 814 g/

m^2 as the minimum reported biomass (Parsons and Stohlgren 1986) and 990 g/m^2 as the maximum reported biomass (Stohlgren et al. 1989). Of the species reported, *Q. berberidifolia* had slightly higher leaf biomass compared to *A. fasciculatum* (310 g/m^2 compared to 247 g/m^2), and *C. cuneatus* the least (47 g/m^2). *Q. berberidifolia* also had the highest stem biomass and litter biomass, although litter biomass at the species level was only available for *Q. berberidifolia* and *C. greggii* (Table 4).

Patterns of aboveground biomass with precipitation. The 36 plots we mapped ranged from coastal locations in Ventura County to higher elevation areas in the Angeles National Forest. It is these higher elevation areas where mean annual precipitation is also highest (Fig. 4). The correlation of mean aboveground biomass with mean annual precipitation showed a positive relationship with biomass increasing with greater mean precipitation ($R^2 = 0.23$, Fig. 5).

Belowground Biomass Estimates

Belowground biomass was by far the least reported metric in the literature. Only four studies reported belowground biomass values determined via direct measurements. Two were published in the late 1970's, one in the early 1980's and one in 2004, all in southern California. The most detailed studies were

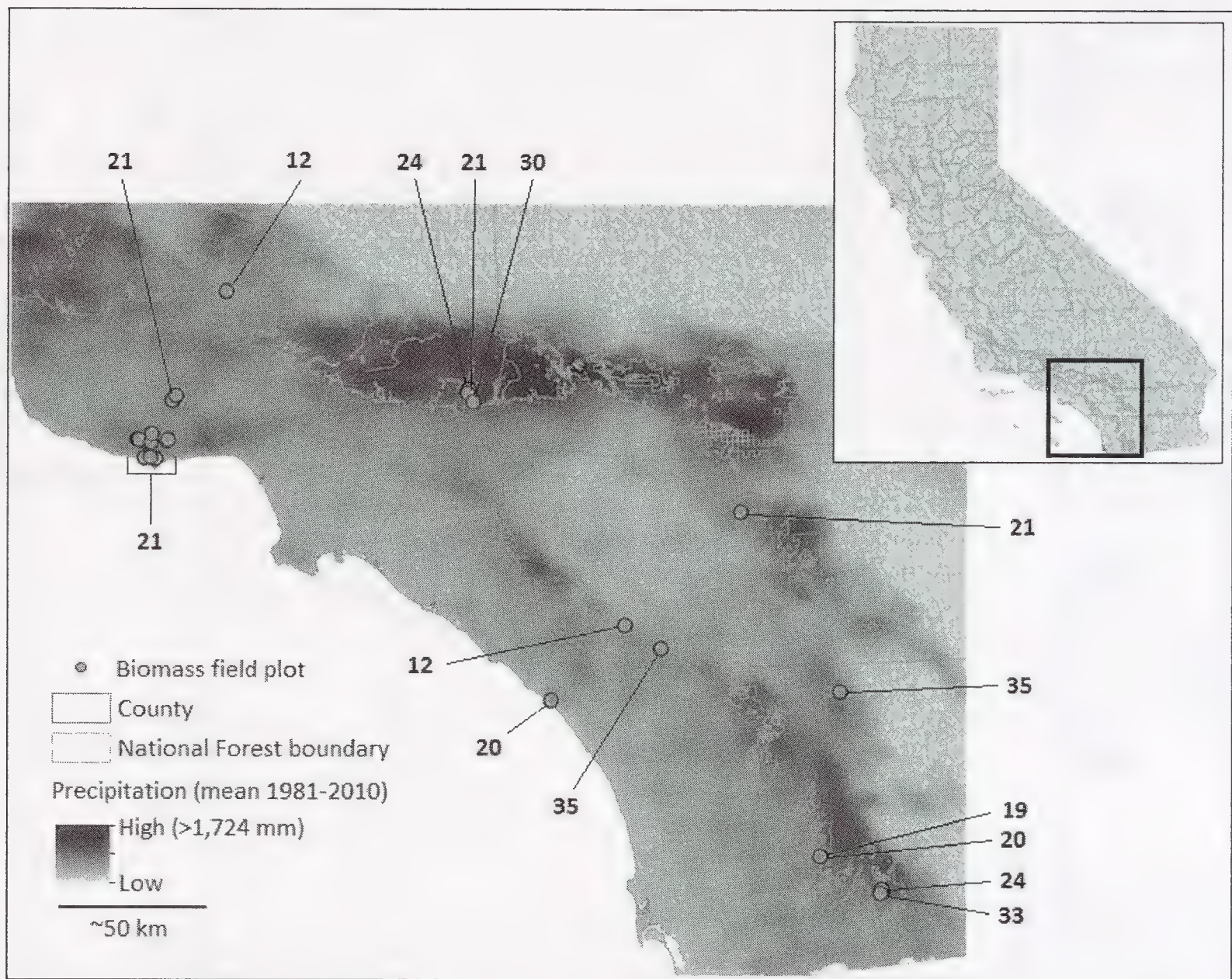


FIG. 4. Location of 36 field plots mapped with mean annual precipitation. Inset map shows enlarged area outlined in black. Numbered plot locations correspond to references listed in Appendix 1. Note: (a) a number of plots in the map overlap making them difficult to distinguish and (b) plots with multiple stand values were averaged where stands were >10 yr olds.

undertaken by Kummerow et al. (1977) and Miller and Ng (1977). In a 70 m² mixed chaparral stand dominated by *A. fasciculatum*, Kummerow et al. (1977) wired shrubs into place and hydraulically excavated the roots. Biomass as well as root:shoot

ratios were determined for each individual shrub excavated and belowground biomass values ranged from about 1402 g/m² to 4789 g/m² for *A. fasciculatum*, 2028 g/m² for *Arctostaphylos pungens*, and 1442 g/m² for *Ceanothus greggii*. An average

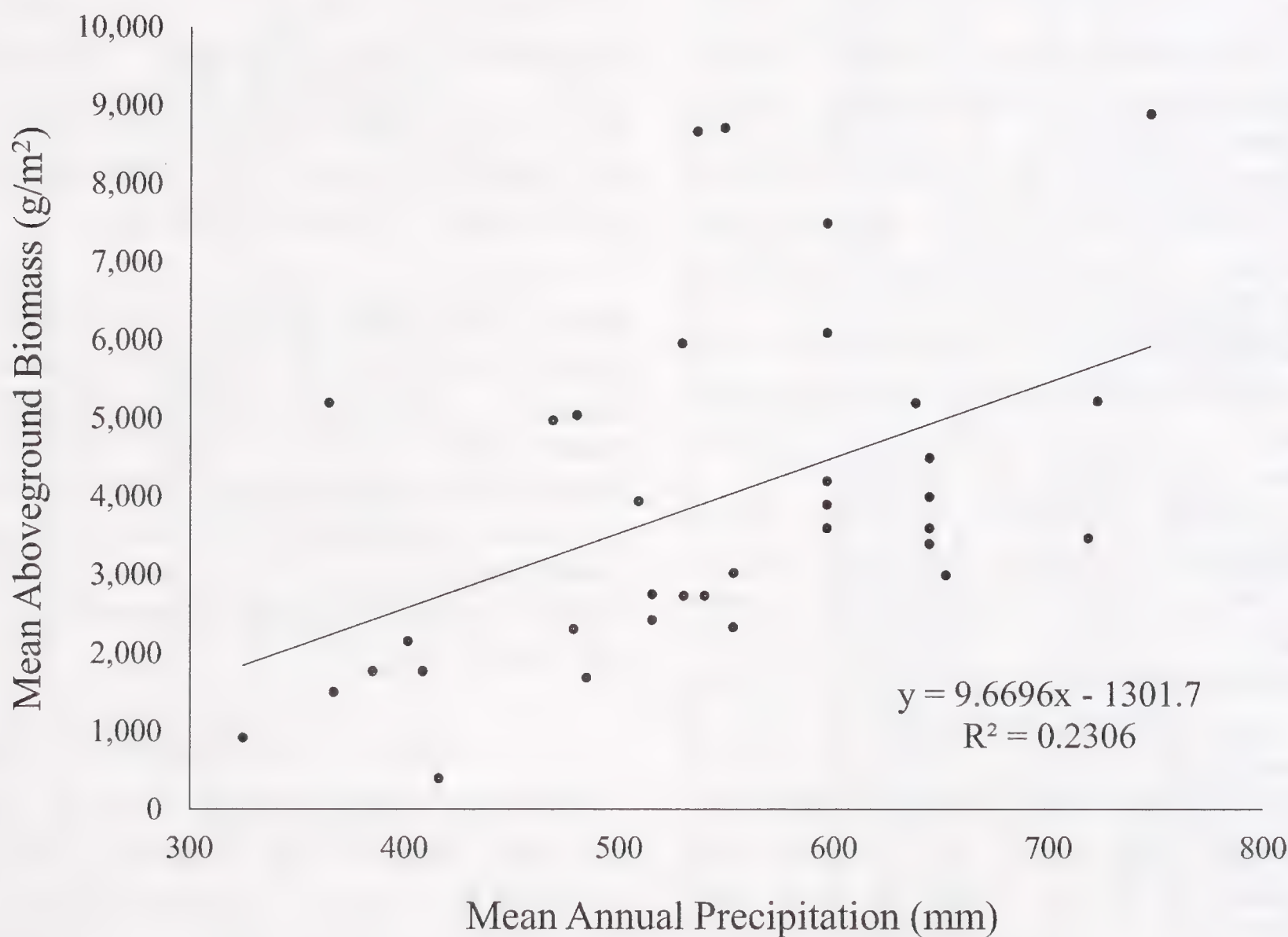


FIG. 5. Correlation between mean aboveground biomass and mean annual precipitation (1981–2010) for 36 field plots with precise coordinates.

overall root:shoot ratio of 0.6 was determined for all species included in the study. Species-specific root:shoot ratios included 0.44 for an *Arctostaphylos pungens* individual and 0.7 for an *A. fasciculatum* individual (Kummerow et al. 1977). Similarly, Miller and Ng (1977) hydraulically excavated individual shrubs in a 21 yr old mixed chaparral stand. Biomass values reported were per shrub only, however, and varied greatly depending on shrub size. *A. fasciculatum* was the main shrub analyzed in the study (4 individuals total) and had a root:shoot ratio ranging from 0.49 to 0.69.

Lipson et al. (2004) undertook a study of the effects of long-term elevated atmospheric CO₂ on root growth among other variables in an 11 yr old *A. fasciculatum* dominated stand. Samples were collected for root biomass measurements using a 10 cm diameter metal soil coring device to a depth of 30 cm, and reported a root biomass of 250 m/g² in the control, which is likely an underestimate of the total belowground biomass due to sampling methods. Riggan and Lopez (1982) assessed nitrogen relations in a 35 yr old *Q. berberidifolia* dominated chaparral stand and report the pre-fire burl biomass of *Q. berberidifolia* as 1330 g/m² but did not assess the biomass of the entire root system.

There were also several studies that looked at root:shoot ratios of shrub seedlings (<12 months old) to assess nitrogen fertilization and allocation tradeoffs among life history traits, among other things. Padgett and Aleen (1999), for example, assessed biomass accumulation in coastal sage scrub species after growing for three months in pots and found that *Artemisia californica* root:shoot ratios range from 0.12 to 0.48 depending on the amount and type of nitrogen added. In a study comparing biomass allocation in shrubs exhibiting three different life history traits, Pratt et al. (2012) found that non-sprouting shrubs (e.g., *Ceanothus megacarpus*, *C. cuneatus*) had an average root:shoot ratio of 0.77 when grown in the sun and 0.44 when grown in the shade. Facultative sprouters (e.g., *C. spinosus*, *C. leucodermis*) had an average root:shoot ratio of 0.84 in the sun and 0.31 in the shade and lastly, obligate seeders (e.g., *Rhamnus ilicifolia*, *R. californica*) had an average root:shoot ratio of 1.7 in the sun and 0.89 in the shade (Pratt et al. 2012).

DISCUSSION

Our review included 37 studies over 72 yrs from which we compiled summary estimates on total, live and dead biomass by age class, as well as information on leaf biomass, live and dead stem biomass, and belowground biomass from a subset of these studies. Key findings were that overall total aboveground biomass in the shrub communities reported was greatest in mixed chaparral (3461 g/m²), followed by chamise chaparral (2114 g/m²), and coastal sage scrub with a total aboveground biomass less than half that of mixed chaparral (1583 g/m²). In each

community total average aboveground biomass generally increased with the age of the stand. Other variables measured included leaf biomass, stem biomass and litter biomass which were also all highest in mixed chaparral compared to chamise chaparral or coastal sage scrub communities. By species, *C. greggii* had the highest average aboveground biomass (4877 g/m²) and *C. cuneatus* had the least biomass with 881 g/m². *Q. berberidifolia* had the highest leaf, total stem, and litter biomass of the four species reported and *A. fasciculatum* had the highest belowground biomass of the two species where reporting was possible, with an average root:shoot ratio of 0.6.

Although we summarized available data on the annual net growth of shrubland communities and individual species, we did not include biomass estimates for annual flower and fruit production in our summaries due to the lack of information on this biomass component in the literature as well as the ephemeral nature of reproductive structures. Reproductive output, however, can be quite substantial, especially for non-sprouting shrub species (e.g., over 150 g/m² for *Arctostaphylos glauca*; Keeley and Keeley 1977). While this biomass does not remain as standing biomass on individual shrubs over time, it is likely accounted for indirectly through estimates of biomass present in the litter layer. Annual reproductive output is important to take into consideration but it may not be relevant when estimating long-term carbon storage.

This review provides a summary of biomass at the community and species level, however, levels of biomass vary considerably across the landscape, most notably with water availability and associated plant water stress during the summer drought (Mooney 1977a). Areas with higher annual precipitation and at higher elevations, e.g., mesic north-facing slopes, and slopes with deeper soils, have sustained high rates of production (Riggan and Dunn 1982). The example we provide using the 36 plots which had precise coordinates supports this point, and shows that mean aboveground biomass has a positive correlation with mean annual precipitation in southern California. Miller (1947) estimated that north facing slopes have roughly 30% more water available in the soil, and consequently chaparral communities on these aspects develop a larger leaf area and higher rates of production than those on south facing slopes (Krause and Kummerow 1977).

A notable point from these summaries is the amount of dead aboveground biomass reported. Dead biomass accounts for approximately one-half the total biomass in coastal sage shrub, one-third in chamise chaparral, and one-fifth in mixed chaparral. The ratio of live to dead biomass in a stand is often related to the stand's fire history. Chaparral species typically have one of three life history strategies: obligate seeder, obligate resprouter, or facultative seeder. Of these three strategies, obligate resprouters and facultative seeders are capable of regenerating

vegetatively after fire (Keeley and Keeley 1988). Shrubs that resprout typically have belowground lignotubers, also called burls, that store carbohydrates and send out sprouts when burned. The biomass of these burls can make up a significant portion of the overall biomass of an individual shrub (Kummerow et al. 1977, Miller and Ng 1977). Additionally, recently burned stands tend to have a higher ratio of dead to live aboveground biomass due to the original stems not being fully consumed by the fire. Alternatively, during long fire-free periods, slow decomposition rates can lead to dead material accumulating in these stands and if fire has not entered into a stand for long enough, it is possible for more competitive, disturbance-free taxa to replace disturbance-dependant taxa, altering the type and amount of biomass in the system (Keeley and Keeley 1988, Hilbert and Larigauderie 1990).

Using simulation modeling, Hilbert and Larigauderie (1990) investigated the mechanisms behind stand senescence in Mediterranean-climate shrublands using available data in the literature. The authors developed two versions of a general model based on dynamics observed in pure stands of *Adenostoma fasciculatum*: one that accounts for individual shrub senescence (e.g., accelerated mortality of mature plants) and one that does not. According to the model results, live biomass and cover tends to peak at about 25–30 yr and litter biomass peaks at about 40 yr after fire, while standing dead biomass shows a slow increase over a 100 yr period. With the incorporation of shrub senescence in the model these trends were accelerated. The authors concluded that the two main factors leading to a decline in biomass and cover over time in shrubland stands are the absence of recruitment and the limit of individual shrub growth (Hilbert and Larigauderie 1990).

Studies varied in how biomass values were generated. The majority of studies utilized allometric equations to estimate biomass across a certain area. This method typically involves select whole shrub harvesting to create linear equations which are then used to calculate plot level biomass using stem diameter measurements. Very few of the studies included in this review carried out full plant harvests that included the root system. In general, the use of allometric equations and pre-determined root:shoot ratios appear to be the most popular methods for generating stand-level biomass. Species-level biomass studies, on the other hand, typically select several shrubs rather than a plot area, allowing a more thorough measurement of biomass for each individual. However, these studies are also extremely limited in the number extracting belowground biomass for direct measurements. Sample size and plot size varied widely in the identified literature, depending on the focus of a given study as well as the resources available to undertake it, sample size varied from $n=1$ to $n=100$ and the size of plots varied from 1 m^2 (Guo 2001) to 120 m^2 (Riggan et al. 1988). This is important to recognize as both the number of

samples and the size of plots influence the amount of variation captured within a shrubland community. A larger plot (especially one that captures different slopes and aspects), for example, will likely capture openings within the shrubland as well as both live and dead shrubs, providing biomass values that are more representative across the landscape.

Of particular note in undertaking this review is the comparative lack of studies published in the last 10 yr. The majority of the field studies (60%) were carried out in the 1970's and 1980's, a time at which there was funding and great academic and practical interest in comparing the five Mediterranean-type climate regions of the world. For example, the United Nations Man and the Biosphere Programme (MAB) was launched in the early 1970's as an intergovernmental scientific program aiming to promote planning and implementation of research and training programs (<http://www.unesco.org/new/en/natural-sciences/environment/ecological-sciences/man-and-biosphere-programme/>). As part of this program, in 1976 the San Dimas Experimental Forest (designated in 1933) in the San Gabriel Mountains of southern California was established as a biosphere reserve, which resulted in numerous experimental studies in chaparral landscapes. In the 1970s the US also joined the International Biological Program (IBP) which focused on documenting the productive capacity of different regions of the world. More specifically, as part of the IBP, a Convergent Evolution program was created to compare the productive structure of vegetation that evolved to meet the challenges of dry summer climates, for example between California and Chile (Mooney and Dunn 1970, Mooney 1977b). Additionally, the relative lack of more recent field studies, especially those that assess belowground biomass, may be due to a shifting focus towards using remote sensing tools to estimate biomass at much larger scales. These studies typically rely on previously established field-based biomass estimates and are often focused on making repeatable regional biomass estimates, often disregarding the importance of belowground biomass (e.g., Schmidt et al. 2016, Uyeda et al. 2017). While it is important to continue developing the most accurate and efficient methods for determining biomass at a landscape level, further work quantifying belowground biomass would strengthen our understanding of biomass accumulation in shrublands.

In this review, by parsing out biomass at the stand and species level and reporting on above- and belowground biomass, live and dead biomass, and leaf, litter and stem biomass, we provide a valuable resource for land managers and conservation practitioners who need estimates of shrubland biomass at different life stages. Understanding these biomass values is foundational information for mapping biomass and monitoring changes, and ultimately quantifying carbon storage ecosystem services (it is generally assumed that carbon makes up 45–50% of

plant biomass; Kort and Turnock 2003, Schlesinger and Bernhardt 2013).

While much attention has focused on the role of forested landscapes as a sink of atmospheric CO₂, the contribution of Mediterranean climate shrublands to the global carbon cycle has received little attention (Evrendilek et al. 2006). Despite shrublands historically being considered overmature after reaching 60 yrs of age (Hanes 1971), shrublands over 100 yrs old have been reported as vigorous (Keeley 1992). Indeed, Luo et al. (2007) recorded a 100 yr old chamise-dominated chaparral stand to be a significant carbon sink (-155 g C/m²/yr). In another study of Mediterranean climate shrublands in Italy, shrublands were reported to remove significant quantities of C from the atmosphere (2200 g C/m²/yr; Gratani et al. 2013). Future changes in climate are likely to impact the capability of shrublands to store and sequester carbon, however there is considerable uncertainty surrounding this. For example, some studies predict that changes in temperature and precipitation may decrease the area of shrublands in the North American Mediterranean Climate Zone owing to expansion of grasslands (Hayhoe et al. 2004) while other studies predict an increase as shrublands encroach into areas currently dominated by conifers in California (Lenihan et al. 2008). In addition, climate changes may lead to variations of shrubland structure and productivity (Haase et al. 2000) and consequently effect carbon sequestration and storage capabilities (Evrendilek et al. 2006). Luo et al. (2007), for example, recorded that after a period of severe drought the ability of the chaparral stand to sequester carbon was limited. Through increasing our knowledge surrounding biomass accumulation and associated carbon sequestration in shrublands, land managers will be better able to understand the potential for these stands to sequester carbon in the future and also provide the information necessary for embarking on Payment for Ecosystem Services schemes for carbon.

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APPENDIX 1. REFERENCES FOR LITERATURE INCLUDED IN SUMMARY TABLES. Table 1. References: 1–7, 9–12, 17, 20–25, 27, 28, 32–36. Table 2. References: 1–3, 5, 10, 12, 17, 21, 23, 27, 30, 31, 35. Table 3. References: 2, 3, 5, 7, 8, 10, 14, 21, 35, 37. Table 4. References: 4, 7, 9, 12, 13, 15, 16, 18–20, 22, 25–32, 35.

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APPENDIX 2. REPORTED ABOVEGROUND BIOMASS VALUES ORGANIZED BY COMMUNITY TYPE, GENERAL LOCATION, AND AGE OF STAND. Studies that did not report on total, live or dead aboveground biomass are excluded from this table. When a study provided multiple biomass values for the same age and location, we report the average of those values. ^aGeneral locations are based on descriptions provided in the reference text, for more detailed information please refer to the original source; ^bReferences are listed in Appendix 1.

Community type	General location ^a	Age (yrs)	Aboveground biomass (g/m ²)			Reference ^b
			Total	Live	Dead	
Mixed chaparral	Los Padres NF	5	—	2055	—	28
Mixed chaparral	Los Padres NF	12	—	3232	—	28
Mixed chaparral	Los Padres NF	21	6337	4871	1466	28
Mixed chaparral	Los Padres NF	25	3039	2012	1028	4
Mixed chaparral	San Dimas Experimental Forest, Angeles NF	6	1430	1330	100	24
Mixed chaparral	San Dimas Experimental Forest, Angeles NF	11	2817	2638	179	34
Mixed chaparral	San Dimas Experimental Forest, Angeles NF	21	5575	4838	743	24
Mixed chaparral	Angeles NF	33	8900	6008	2892	21
Mixed chaparral	Sky Oaks Biological Field Sta., San Diego Co.	1	—	200	—	35
Mixed chaparral	Sky Oaks Biological Field Sta., San Diego Co.	2	—	475	—	35
Mixed chaparral	Sky Oaks Biological Field Sta., San Diego Co.	3	—	660	—	35
Mixed chaparral	Sky Oaks Biological Field Sta., San Diego Co.	4	—	600	—	35
Mixed chaparral	Sky Oaks Biological Field Sta., San Diego Co.	5	—	500	—	35
Mixed chaparral	Sky Oaks Biological Field Sta., San Diego Co.	6	—	560	—	35
Mixed chaparral	Sky Oaks Biological Field Sta., San Diego Co.	7	—	700	—	35
Mixed chaparral	Sky Oaks Biological Field Sta., San Diego Co.	8	—	800	—	35
Mixed chaparral	Sky Oaks Biological Field Sta., San Diego Co.	54	3005	—	—	17
Mixed chaparral	Sky Oaks Biological Field Sta., San Diego Co.	85	3085	—	—	17
Mixed chaparral	Cleveland NF; Sky Oaks Biological Sta.; West of Anza-Borrego Desert	4	1500	800	700	1
Mixed chaparral	Cleveland NF; Sky Oaks Biological Sta.; West of Anza-Borrego Desert	11	2600	2400	200	1
Mixed chaparral	Cleveland NF; Sky Oaks Biological Sta.; West of Anza-Borrego Desert	54	5000	3700	1300	1
Mixed chaparral	Cleveland NF; Sky Oaks Biological Sta.; West of Anza-Borrego Desert	80	4500	3500	1000	1
Mixed chaparral	Cleveland NF; Sky Oaks Biological Sta.; West of Anza-Borrego Desert	85	4500	3700	800	1
Mixed chaparral	Descanso Ranger District, Cleveland NF	2	1813	—	—	36
Mixed chaparral	Descanso Ranger District, Cleveland NF	3	199	—	—	36
Mixed chaparral	Descanso Ranger District, Cleveland NF	5	540	—	—	36
Mixed chaparral	Descanso Ranger District, Cleveland NF	10	1489	—	—	36
Mixed chaparral	Descanso Ranger District, Cleveland NF	11	411	—	—	36
Mixed chaparral	Descanso Ranger District, Cleveland NF	13	1639	—	—	36
Mixed chaparral	Descanso Ranger District, Cleveland NF	18	1839	—	—	36
Mixed chaparral	Descanso Ranger District, Cleveland NF	20	1418	—	—	36
Mixed chaparral	Descanso Ranger District, Cleveland NF	22	2813	—	—	36
Mixed chaparral	Kitchen Creek, Cleveland NF	1	—	280	—	24
Mixed chaparral	Kitchen Creek, Cleveland NF	7	1320	—	—	33
Mixed chaparral	Kitchen Creek, Cleveland NF	28	4140	—	—	33
Mixed chaparral	Kitchen Creek, Cleveland NF	35	—	2325	—	25
Mixed chaparral	Kitchen Creek, Cleveland NF	35	3000	3000	0	24
Mixed chaparral	Kitchen Creek, Cleveland NF	68	5060	—	—	33
Mixed chaparral	Camp Pendleton, San Diego Co.	—	6236	3853	890	2
Mixed chaparral	Camp Pendleton, San Diego Co.	—	9774	—	—	3
Mixed chaparral	Camp Pendleton, San Diego Co.	“mature”	9000	4500	4500	6
Mixed chaparral	Puerta La Cruz Rd, Warner Springs, San Diego Co.	4	840	—	—	17
Mixed chaparral	Puerta La Cruz Rd, Warner Springs, San Diego Co.	11	1873	—	—	17
Mixed chaparral	Puerta La Cruz Rd, Warner Springs, San Diego Co.	54	2535	—	—	17
Mixed chaparral	Puerta La Cruz Rd, Warner Springs, San Diego Co.	80	3500	—	—	17
Mixed chaparral	Echo Valley International Biological Program	23	2308	—	—	20
Mixed chaparral	Leo Carrillo State Park, Santa Monica Mountains, Los Angeles Co.	22	7705	6482	1223	7
Mixed chaparral	Stunt Ranch Santa Monica Mountains UC Reserve, Los Angeles Co.	1	130	—	—	11
Mixed chaparral	Stunt Ranch Santa Monica Mountains UC Reserve, Los Angeles Co.	2	410	—	—	11

APPENDIX 2. CONTINUED

Community type	General location ^a	Age (yrs)	Aboveground biomass (g/m ²)			Reference ^b
			Total	Live	Dead	
Mixed chaparral	Stunt Ranch Santa Monica Mountains UC Reserve, Los Angeles Co.	3	590	–	–	11
Mixed chaparral	Stunt Ranch Santa Monica Mountains UC Reserve, Los Angeles Co.	4	855	–	–	11
Mixed chaparral	Santa Monica Mountains National Recreation Area, Los Angeles Co.	14	5010	3945	1065	21
Mixed chaparral	Santa Monica Mountains National Recreation Area, Los Angeles Co.	17	8730	7420	1310	5
Mixed chaparral	Santa Monica Mountains National Recreation Area, Los Angeles Co.	17	8698	7420	1255	21
Mixed chaparral	Santa Monica Mountains National Recreation Area, Los Angeles Co.	18	5920	4840	1080	5
Mixed chaparral	Santa Monica Mountains National Recreation Area, Los Angeles Co.	18	5963	4864	1098	21
Mixed chaparral	North Mountain Experimental Area, Riverside Co.	"mature"	7846	6165	1681	9
Mixed chaparral	Bear Creek, Riverside Co.	30-50	5201	4775	426	12
Mixed chaparral	Sequoia National Park, Tulare Co.	3	540	–	–	32
Mixed chaparral	Sequoia National Park, Tulare Co.	15	3020	–	–	32
Mixed chaparral	Sequoia National Park, Tulare Co.	24	3165	–	–	22
Mixed chaparral	Southern California	14	4900	–	–	23
Mixed chaparral	Southern California	19	7300	–	–	23
Mixed chaparral	Southern California	33	8200	–	–	23
Mixed chaparral	Southern California	50	5100	–	–	23
Mixed chaparral	Southern California	55	11800	–	–	23
Mixed chaparral	Mendocino Co.; Lake Co.; Shasta Co.	1	127	–	–	27
Mixed chaparral	Mendocino Co.; Lake Co.; Shasta Co.	2	204	–	–	27
Mixed chaparral	Mendocino Co.; Lake Co.; Shasta Co.	3	433	–	–	27
Mixed chaparral	Mendocino Co.; Lake Co.; Shasta Co.	4	629	–	–	27
Mixed chaparral	Mendocino Co.; Lake Co.; Shasta Co.	5	1011	–	–	27
Mixed chaparral	Mendocino Co.; Lake Co.; Shasta Co.	6	1088	–	–	27
Mixed chaparral	Mendocino Co.; Lake Co.; Shasta Co.	7	1134	–	–	27
Mixed chaparral	Mendocino Co.; Lake Co.; Shasta Co.	8	1186	–	–	27
Mixed chaparral	Mendocino Co.; Lake Co.; Shasta Co.	"Old stand"	3100	–	–	27
Chamise chaparral	San Dimas Experimental Forest, Angeles NF	1	273	115	158	30
Chamise chaparral	San Dimas Experimental Forest, Angeles NF	3	1087	521	566	30
Chamise chaparral	San Dimas Experimental Forest, Angeles NF	9	–	863	–	30
Chamise chaparral	San Dimas Experimental Forest, Angeles NF	18	2039	1659	380	30
Chamise chaparral	San Dimas Experimental Forest, Angeles NF	37	4909	2726	2184	30
Chamise chaparral	Sky Oaks Biological Field Sta., San Diego Co.	"pre-fire"	–	1686	–	35
Chamise chaparral	Cleveland NF; Sky Oaks Biological Sta.; West of Anza-Borrego Desert	4	1300	800	500	1
Chamise chaparral	Cleveland NF; Sky Oaks Biological Sta.; West of Anza-Borrego Desert	11	1440	1400	40	1
Chamise chaparral	Cleveland NF; Sky Oaks Biological Sta.; West of Anza-Borrego Desert	54	2050	1800	250	1
Chamise chaparral	Cleveland NF; Sky Oaks Biological Sta.; West of Anza-Borrego Desert	80	1600	1400	200	1
Chamise chaparral	Cleveland NF; Sky Oaks Biological Sta.; West of Anza-Borrego Desert	85	3400	2500	900	1
Chamise chaparral	Camp Pendleton, San Diego Co.	–	2843	1189	1015	2
Chamise chaparral	Camp Pendleton, San Diego Co.	–	3755	–	–	3
Chamise chaparral	Puerta La Cruz Rd, Warner Springs, San Diego Co.	4	770	–	–	17
Chamise chaparral	Puerta La Cruz Rd, Warner Springs, San Diego Co.	80	1510	–	–	17
Chamise chaparral	Santa Monica Mountains National Recreation Area, Los Angeles Co.	13	2805	2085	720	5
Chamise chaparral	Santa Monica Mountains National Recreation Area, Los Angeles Co.	13	2679	2074	605	21
Chamise chaparral	Santa Monica Mountains National Recreation Area, Los Angeles Co.	14	2309	1547	762	21
Chamise chaparral	Santa Monica Mountains National Recreation Area, Los Angeles Co.	17	3050	2170	880	5

APPENDIX 2. CONTINUED

Community type	General location ^a	Age (yrs)	Aboveground biomass (g/m ²)			Reference ^b
			Total	Live	Dead	
Chamise chaparral	Santa Monica Mountains National Recreation Area, Los Angeles Co.	17	2735	2197	538	21
Chamise chaparral	Santa Monica Mountains National Recreation Area, Los Angeles Co.	40	2760	2220	540	5
Chamise chaparral	Santa Monica Mountains National Recreation Area, Los Angeles Co.	40	2735	2219	516	21
Chamise chaparral	Newhall, Los Angeles Co.	35-50	1771	1569	202	12
Chamise chaparral	San Bernardino NF	55	3945	3049	897	21
Chamise chaparral	Santa Rosa Plateau Preserve, Riverside Co.	10	1636	1054	583	12
Chamise chaparral	Sequoia National Park, Tulare Co.	>60	3066	—	—	31
Chamise chaparral	Southern California	10	1600	—	—	23
Chamise chaparral	Southern California	14	2300	—	—	23
Chamise chaparral	Southern California	19	2800	—	—	23
Chamise chaparral	Southern California	50	1700	—	—	23
Chamise chaparral	Southern California	55	4000	—	—	23
Chamise chaparral	Mendocino Co.; Lake Co.; Shasta Co.	1	175	—	—	27
Chamise chaparral	Mendocino Co.; Lake Co.; Shasta Co.	2	309	—	—	27
Chamise chaparral	Mendocino Co.; Lake Co.; Shasta Co.	3	465	—	—	27
Chamise chaparral	Mendocino Co.; Lake Co.; Shasta Co.	4	740	—	—	27
Chamise chaparral	Mendocino Co.; Lake Co.; Shasta Co.	5	1000	—	—	27
Chamise chaparral	Mendocino Co.; Lake Co.; Shasta Co.	6	1130	—	—	27
Chamise chaparral	Mendocino Co.; Lake Co.; Shasta Co.	7	1215	—	—	27
Chamise chaparral	Mendocino Co.; Lake Co.; Shasta Co.	8	1276	—	—	27
Coastal sage scrub	Camp Pendleton, San Diego Co.	—	2869	661	773	2
Coastal sage scrub	Camp Pendleton, San Diego Co.	—	2959	—	—	3
Coastal sage scrub	Santa Margarita Ecological Reserve, San Diego Co.	1	—	575	—	35
Coastal sage scrub	Santa Margarita Ecological Reserve, San Diego Co.	2	—	395	—	35
Coastal sage scrub	Santa Margarita Ecological Reserve, San Diego Co.	3	—	630	—	35
Coastal sage scrub	Santa Margarita Ecological Reserve, San Diego Co.	4	—	520	—	35
Coastal sage scrub	Santa Margarita Ecological Reserve, San Diego Co.	5	—	200	—	35
Coastal sage scrub	Santa Margarita Ecological Reserve, San Diego Co.	6	—	210	—	35
Coastal sage scrub	Santa Margarita Ecological Reserve, San Diego Co.	7	—	325	—	35
Coastal sage scrub	Santa Margarita Ecological Reserve, San Diego Co.	8	—	420	—	35
Coastal sage scrub	Santa Margarita Ecological Reserve, San Diego Co.	35	—	400	—	35
Coastal sage scrub	Santa Monica Mountains National Recreation Area, Los Angeles Co.	14	1750	1170	580	5
Coastal sage scrub	Santa Monica Mountains National Recreation Area, Los Angeles Co.	14	1771	1188	583	21
Coastal sage scrub	Santa Monica Mountains National Recreation Area, Los Angeles Co.	18	2152	1300	852	21
Coastal sage scrub	Santa Monica Mountains National Recreation Area, Los Angeles Co.	30	1502	986	516	21
Coastal sage scrub	Santa Monica Mountains, Los Angeles Co.	1	598	—	—	14
Coastal sage scrub	Leo Carrillo State Park, Santa Monica Mountains	22	1172	925	247	7
Coastal sage scrub	Leo Carrillo State Park, Santa Monica Mountains	22	1418	—	—	8
Coastal sage scrub	Robert J. Bernard Biological Field Sta., Los Angeles Co.	40	2725	—	—	37

COYOTE BRUSH AS FACILITATOR OF NATIVE CALIFORNIA PLANT RECOVERY IN THE SANTA MONICA MOUNTAINS

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ABSTRACT

Exotic annual grasses now cover large areas of southern California that were once stands of native California sage scrub (CSS), or a mixture of native grasses, forbs, and CSS. Both CSS and California grasslands are threatened habitats, where restorations of type-converted landscapes are often burdened by the persistent dominance of non-native annual grasses. Research finds that once exotic grasses take hold in these areas, native plant communities are extremely slow to recover, if they recover at all. Coyote brush (*Baccharis pilularis* DC) is a native shrub common to CSS habitat and often appears in a complex mosaic with other vegetation types including grasslands. Coyote brush has been documented invading grasslands, resulting in a change of state from grassland to shrubland in northern California. This study investigates the long-term consequences of coyote brush invasion in a type-converted landscape of southern California. Stands of expanding coyote brush were transected to identify species composition along a spatial and temporal continuum. Results show that, following initial invasion, non-native species are gradually replaced by, not only coyote brush, but also several other noteworthy native species. This study finds that over the 37 yr timeframe, exotic grasses gradually decline while native plant cover increases in landscapes invaded by coyote brush. We conclude that in the Santa Monica Mountain areas studied, coyote brush invasion of type-converted landscapes leads to increased native plant diversity that includes native grasses and a variety of shrubs.

Key Words: *Baccharis pilularis*, California sage scrub (CSS), coastal sage scrub, facilitation, native bunchgrasses, passive restoration.

Exotic annual grasses now cover large areas of southern California that were once stands of native California sage scrub (CSS), or a mixture of CSS and native perennial grasses and forbs (Westman 1981; Freudenberger et al. 1987; Minnich 2008). These landscapes were invaded and replaced by exotic grasses of mostly European origin (Burcham 1957; Stromberg et al. 2007; Minnich 2008). Numerous studies find that once exotic grasses take hold in these areas, native plant communities are extremely slow to recover, if they recover at all (Hobbs 1983; Davis 1994; Styliniski and Allen 1999; Engelberg et al. 2013). In part, this is because invasive grasses are competitive with native species, as experimental studies have shown, and thus grass control is necessary before natives can reestablish (Eliason and Allen 1997; Gillespie and Allen 2004). Fragments of coastal sage scrub or native bunch grasses that do remain are often intermixed with persistent stands of exotic grasses (e.g., Freudenberger et al. 1987), even after removal of disturbances that promote grass colonization, such as grazing, frequent fire, or mechanical disking (Davis 1994; Zink et al. 1995; Engelberg et al. 2013; Laris et al. 2017).

In cases where CSS shrubs have recovered in areas formerly type-converted to exotic grasses, the diver-

sity of shrub species is often lower than neighboring existing stands of CSS. This has been particularly true in instances where areas formerly dominated by CSS, which were forcibly converted to grassland by mechanical means, eventually recovered after release from intensive disturbance (Davis 1994; Styliniski and Allen 1999; Engelberg et al. 2013; Laris et al. 2017). In some cases, a single species, such as coyote brush (*Baccharis pilularis*), becomes a dominant monotypic shrub cover at least temporarily (Laris et al. 2017). Indeed, coyote brush is one of few CSS species that has proven capable of readily (re)colonizing landscapes invaded by exotic annual grasses (Fig. 1, McBride 1974; Williams and Hobbs 1989; Zavaleta and Kettley 2006).

In spite of the fact that coyote brush is a native shrub common in coastal areas and other locales, its propensity to colonize and expand into areas forming monotypic stands has been the subject of some debate in terms of its perceived pros and cons by different land management agents. From the perspective of some managers of California State Park lands, for example, who view the grasslands as historical relics of a lost landscape, the coyote brush invasion is seen as a negative change. Contrastingly, many conservationists view coyote brush expansion



FIG. 1. Coyote brush-annual grassland boundary. Note the dense band of coyote brush and individuals expanding into the grassland (Photograph by Paul Laris).

in a positive light primarily because it is a *native* shrub expanding into largely *exotic* grasslands. In the first case the vegetation form—grassland—is deemed most critical, while in the latter case it is the vegetation origin—nativeness—that matters most (Laris et al. 2017).

In part the debate over the role of coyote brush invasion is due to the fact that the “original” vegetation cover of exotic grasslands remains uncertain. Until relatively recently, it was believed that prior to exotic grass invasion, the valleys and slopes of coastal California mountains and foothills were covered primarily by native California bunch grasses (e.g., *Stipa*) (Clements 1934; Burcham 1957; Barry 1972; Heady 1977). However, recent research casts doubt on this so-called “bunch-grass” hypothesis by arguing that forbs were predominant prior to the Colombian exchange (Hamilton 1997; Schiffman 2005; Minnich 2008). As such, a shift from grassland to a coyote brush shrubland constituted a type conversion in its own right (Russell and McBride 2003), suggesting the landscape may be governed by multiple states and transitions. Still others argue that CSS shrubs were common in areas now covered by exotic grasses and that grazing management practices, which included mechanical disking, primarily caused the type conversion (Laris et al. 2017).

Coyote brush has a number of key traits that give it advantages for rapidly expanding into grasslands. First, wind dispersal, in particular, may give it an advantage over many CSS shrubs, which lack a similar dispersal mechanism (DeSimone and Zedler 2001; Steinberg, 2002). Second, *B. pilularis* grows rapidly and after only two to three yr forms a closed canopy that can shade out exotic grasses. Third, and most importantly perhaps, *B. pilularis* stands are known to provide excellent refuge for small mammals, such as rabbits and rodents, which find safe cover under the shrub canopy and consume exotic grasses and seed. Feeding activity of these small animals is concentrated in grassland areas immedi-

ately adjacent to stands of shrubs and beneath the shrub canopy. The adjacent annual grassland provides poor cover for most of these animals, yet furnishes an excellent food supply for grazers and seed eaters. The activity can be so intense as to create trails of bare soil along the grass/shrub border, which may facilitate additional shrub establishment (Bartholomew 1970; Halligan 1973, 1974). As such, herbaceous-seed dispersal into and seedling survival in *B. pilularis* stands is very low (Hobbs and Mooney 1986), as small rodents and birds consume most developing herbaceous seedlings, perhaps facilitating a shrub domination (Bartholomew 1970; Christensen and Muller 1975; Hobbs and Mooney 1986; DeSimone and Zedler 2001).

It has been well documented that exotic grasses suppress the recovery of native CSS plants by outcompeting juvenile shrubs for resources (Eliason and Allen 1997; Cox and Allen 2008; Fleming et al. 2009). By harboring small mammals that reduce the competition from exotic grasses, *B. pilularis* appears to facilitate conversion of grasslands to dense coyote brush stands (DaSilva and Bartolome 1984). Indeed, Hobbs and Mooney (1986) found that abundances of all herbaceous species declined greatly after *Baccharis* formed a closed canopy, usually within two to three yr of colonizing grassland. At this point, little seed of herbaceous species was dispersed into shrub stands or stored in the soil.

Importantly, *B. pilularis* is thought to have a relatively short lifespan (Hobbs and Mooney 1986). In addition, the coyote brush canopy, which is dense and closed during the plant’s youth, can become progressively open over time as the plant ages. After ten yr or more, the canopy of coyote brush may be sufficiently fragmented such that herbaceous seedlings are able to establish (Fig. 2). It is possible, therefore, that coyote brush could facilitate a recovery of a more diverse mixture of species on the landscape by removing or damping the competition from exotic grasses—a phenomenon appropriately dubbed the “*Baccharis* hypothesis” by DeSimone and Zedler (2001).

In one of the few well-documented long-term studies of coyote brush invasion of a grassland, McBride (1974) found that the coyote brush colonization was a step in the succession of exotic grassland to woodland. McBride found that *Quercus agrifolia* and *Umbellularia californica* became established as seedlings under the canopy of coyote brush. In a related study, Zavaleta and Kettley (2006) found herbaceous biomass and competition from grasses strongly influenced the establishment of woody plants in grassland. They found that progressive increase in *B. pilularis* stand age and decline in herbaceous understory biomass likely increased the probability of successful oak recruitment under older shrubs. It is noteworthy that their study area was on San Francisco Bay Area lands formerly subjected to grazing and mechanical disturbance.

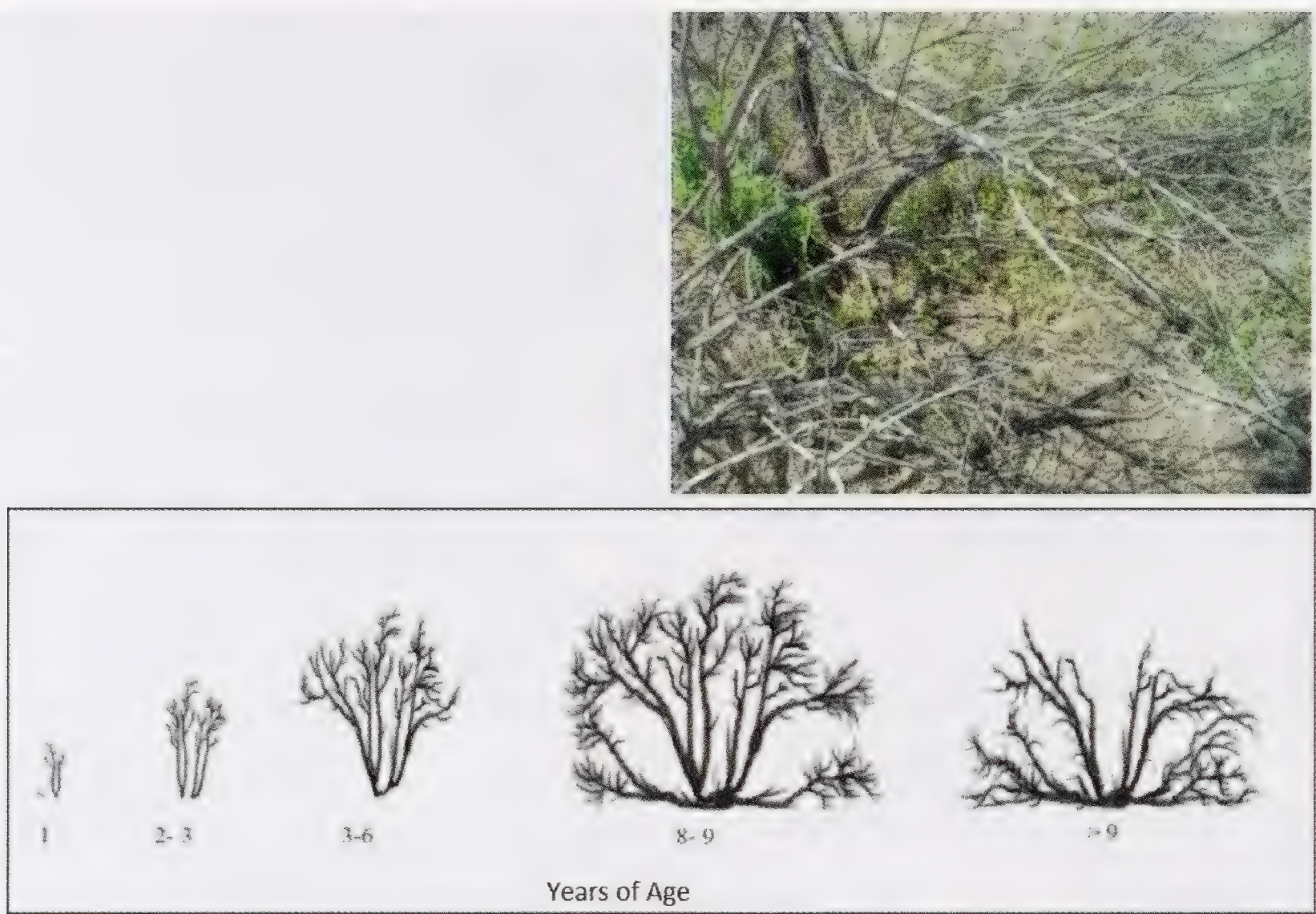


FIG. 2. Photograph of seedlings sprouting beneath a mature coyote brush canopy. Illustration of coyote brush structure over time [reproduced from Hobbs and Mooney (1986)] and Note the opening of the canopy occurs in more mature stands (Photograph by Paul Laris).

To our knowledge, the phenomenon of coyote brush invasion has not been studied systematically in southern California. To explore the possibility that coyote brush plays a facilitating role in native CSS shrubland reestablishment in southern California, and test the *Baccharis pilularis* hypothesis, we sampled plots of coyote brush known to have recently colonized exotic grasslands at numerous sites in the Santa Monica Mountains. We hypothesized that the relative frequency of native plants (shrubs and grasses) would increase, and exotic cover decrease, over time in areas covered by coyote brush stands. We sampled coyote brush stands of known age classes and then compared the results between age classes to determine to effect of coyote brush on native plant establishment over time.

METHODS

Study Area

The Santa Monica Mountains are one of the Transverse Ranges of southern California. They directly border the California Bight from Point Mugu on the west to Pacific Palisades on the coast to the east and continuing eastward inland to the Hollywood Hills. They constitute an anticline thrust southward along the Malibu/Santa Monica fault zone running along the coast. The study areas lie in the western Santa Monica Mountains and adjacent sections of the Simi Hills to the north (Fig. 3).

Miocene Conejo volcanics and lava intrusions are exposed along the western and central core with Miocene mostly marine sedimentary rocks to the

north and south (e.g., Vaqueros, Topanga Canyon, and Modelo formations). Peak elevations in the western Santa Monica Mountains are taller than found in the eastern Santa Monica Mountains, ranging from roughly 400 m to over 900 m. The general east-west trend of the range is interrupted by a few major north-south trending canyons, such as Malibu Canyon, Sepulveda Canyon, and Cahuenga Canyon. Small valleys and canyons are found throughout, making for a complex and high-relief landscape. Soils vary dramatically with elevation, slope, underlying geological substrate, and weathering (Yerkes and Campbell 2005).

The climate is Mediterranean in character, with the warm summer variant along the south-facing slopes along the beach and the hot summer on the north-facing slopes inland. Prevailing air and winter storm circulation is generally from the west, occasionally disrupted by Santa Ana foehn winds from the northeast and diurnal onshore and downslope breezes. Higher elevations experience more precipitation annually and during individual storms, through orographic effects. Lower elevations are drier due to less frequent attainment of saturation vapor pressure on the coast, coupled with and intensified by leeward descending circulation inland. The microclimatic pattern, thus, is extremely diverse and changeable over short distances (National Oceanic and Atmospheric Administration 1985; Barbour et al. 1993; Rundel and Gustafson 2005).

Vegetation reflects the climatic, edaphic, topographical, and geological diversity as well as land use history and fire regime. Chaparral dominates at higher elevations and north-facing slopes, often on the steepest slopes with the most skeletal and



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FIG. 3. Study areas in Santa Monica Mountains of southern California.

unstable soils, while California sage scrub is common on lower slopes and drier situations, with a distinctive variant on coastal bluffs. Oak-dominated woodlands are common in canyons and hill slopes with deeper, well-developed and well-drained soils. Grasslands today dominate valleys and lower slopes and terraces, generally with histories of grazing, cultivation and/or mechanical disturbances to reduce shrub cover (Laris et al. 2017). The fire regime for the Santa Monica Mountains varies by location, but there were major fire events in 1973, 1993, and 2013, giving the specific study areas a fire return interval of twenty yr or less. The extent and floristic character of pre-European California grasslands are poorly understood (Minnich 2008) but, whatever their original character, grasslands in the Santa Monica Mountains today are dominated by exotic annual grasses and forbs and exotic perennial bunch grasses.

CSS, the focus of this study, is a sage-scrub community extending from the central California coast through northwestern Baja. CSS is dominated by semiwoody shrub species, and most of the flora is drought-deciduous, with a smaller portion represented by evergreens and succulents. Common shrub species include a number of sage species, such as *Salvia mellifera* Greene (Lamiaceae), *S. leucophylla*

Greene (Lamiaceae), and *S. apiana* Jeps. (Lamiaceae); two species of the composite family (Asteraceae): *Artemisia californica* Less. and *Encelia californica* Nutt.; and two buckwheat species: *Eriogonum fasciculatum* Benth. and *E. cinereum* Benth (Polygonaceae). The evergreen species *Rhus integrifolia* (Nutt.) Benth. & Hook. f. ex Rothr (Anacardiaceae), *Malosma laurina* (Nutt.) Nutt. ex Abrams (Anacardiaceae), and *Heteromeles arbutifolia* (Lindl.) M. Roem. (Rosaceae) are present, too. CSS also supports the Cactaceae succulents *Opuntia littoralis* (Engelm.) Cockerell, *O. oricola* Philbrick, and *Cylindropuntia prolifera* (Engelm.) F.M. Knuth. CSS tends to be a floristically diverse combination of annuals, perennials, and geophytes, although some of the species can dominate large areas (Rundel and Gustafson 2005; Rundel 2007).

Baccharis pilularis, is a native shrub common to the coastal sage scrub (CSS) habitat of California and often appears in a complex mosaic with other vegetation types including grasslands. *Baccharis pilularis* is common in coastal areas and is found occasionally in the interior grassland and shrubland areas (Rundel and Gustafson 2005).



FIG. 4. View from above a *Baccharis pilularis* stand advancement over time based on image analysis for the four dates (Photograph by Sean Brennan).

Site Selection

To assess changes in species composition beneath coyote brush canopy, we selected stands of *B. pilularis* that had advanced over a 37 yr period, into several adjacent type-converted landscapes dominated by exotic grasses. We identified stands of *B. pilularis* using a vegetation map provided by the Santa Monica Mountains National Recreation Area (SMMNRA) that was constructed using 2001 aerial imagery and ground surveys. *Baccharis pilularis* shrub stand extents were then compared to historical imagery from aerial photographs for 2013, 2001, 1989, and 1976 acquired from SMMNRA. Google Earth’s online historical imagery was also used to facilitate the identification of appropriate study sites (Google Earth, Google, Inc., Mountain View, CA).

The SMMNRA vegetation map provided a highly accurate and reliable source of vegetation data down to the species association level. This dataset was provided in a geographic information system (GIS) shapefile format, allowing for stands of *B. pilularis* polygons to be identified separately from all other vegetation association polygons. Polygons, or stands of *B. pilularis* based on 2001 imagery, were then compared to 2013, 1989, and 1976 imagery. Eleven stands showing the greatest expansion of *B. pilularis* and accessibility were identified, then transected to determine species composition (Fig. 4).

A belt transect sampling method was used to determine species composition at each sampling

location along each transect. Classifying transect portions and associated vegetation by age involved visual comparisons with historical imagery in GIS. Each 50 m transect location was entered into GIS using the field-recorded GPS locations. Then, each transect was separately analyzed across the four imagery dates. When viewing transects over 1976 imagery, most transects appeared as entirely open grassland. Portions of transects where shrubs appear for the first time in 1989 were classified as being at least 23 yr of age, and were assigned to the C-group. When viewing transects over 2001 imagery, portions of transects where newer shrubs appear were classified as being between 12 and 23 yr of age, and were assigned to the B-group. Finally, when viewing transects over 2013 imagery, portions of transects where the newest shrubs appear were classified as less than 12 yr of age, and were assigned to the A-group (Fig. 5).

A belt transect sampling method was used to determine species composition for each sampling location along each transect. Classifying transect portions and associated vegetation by age involved visual comparisons with historical imagery in GIS. Each 50 m transect location was entered into ArcGIS using the field recorded Global Positioning Sensor (GPS) locations (ArcGIS, Release 10.1, Environmental Systems Resource Institute, Redlands, CA). Then each transect was separately analyzed across the four imagery dates.



FIG. 5. Schematic of study design showing progression of coyote brush advancement.

Field Sampling

Transect placement began at the shrub stand edge and proceeded 50 m into the shrub stand, perpendicular to the expanding *B. pilularis* stand's boundary with grassland. Data collection began at the zero mark, followed then at each half meter. As a visual aid, a 1 m wide rod was held at its middle, horizontally to the ground, and perpendicular to the belt transect. Any new plant that touched the 1 m wide vertical plane (0.5 m on each side of the data point) would be counted according to species and transect location. This included all vascular vegetation from surface level into the shrub or tree canopy if applicable. If a plant that had been previously identified continued into the next data sampling point or beyond and no new individual plant was identified, "no change" was attributed to that data point. Areas completely void of vegetation received an attribute of "bare". A data point could contain multiple species as well as having more than one of any single species. All exotic grasses were categorized together as non-native grasses, while all native grasses were also categorized together with the exception of *Elymus condensatus*, a large grass that competes with CSS shrubs. In addition, all non-native mustards were also categorized together. There were 101 data points for each of the 11 transects, for a total of 1,111. Each transect was separately entered into a spreadsheet program listing species and counts for each data point. Transect locations were recorded using a GPS logger.

Transects were separately analyzed by age class using a spreadsheet. While all transects were 50 m in length, each individual age class portion along them

uniquely varied in length along each transect. Each datum, or sampling point, typically had more than one data entry along the crossbar set up at that point, and there would be varying numbers of sampling points within each class depending on the distribution of the age classes along that transect. Therefore, each transect was separately analyzed by age class as a percentage of the age class having any one species. Most transects had three age classes, all with varying lengths. Species within each age class were calculated by percentage of age class containing that species. A final tally of the three age classes across all eleven transects was summarized and also calculated as a percentage of age class containing a species.

Statistical Testing

Since the interest here is evaluating whether *B. pilularis* generally facilitates the re-establishment of native species in exotic dominated annual grasslands, our analyses focused on the counts and relative frequencies of *B. pilularis*, other native species, and exotic species as they change over the three age classes for *B. pilularis*. Frequency counts of native species and non-native species in general and then *B. pilularis* in particular, against all other native species and against all non-native species were cross-tabulated by the three age classes. Given that the sample sizes for our three contingency tables ranged from 710 to 1218 and that the smallest observed cell count was 28, we selected Chi-square tests to judge significance ($\alpha = 0.05$) for these three tests. Additionally, we evaluated the frequency of the no change attribute for shrub canopy extents with a Chi-square

TABLE 1. SPECIES COUNTS BY AGE CLASS (A < 12; 12 < B < 23; C < 23 YR OF AGE). * = NON-NATIVE SPECIES

Species	Count	Age class		
		A	B	C
<i>Adenostoma fasciculatum</i> var. <i>fasciculatum</i>	1	0	0	1
<i>Anagallis arvensis</i> *	27	11	11	5
<i>Artemisia californica</i>	130	12	54	64
<i>Astragalus brauntonii</i>	15	2	2	11
<i>Asclepias fascicularis</i>	6	0	3	3
<i>Baccharis pilularis</i>	215	38	85	92
<i>Brassica nigra</i> * or <i>Hirschfeldia incana</i> *	24	6	16	2
<i>Centaurea melitensis</i>	172	108	59	9
<i>Diplacus aurantiacus</i>	51	14	37	0
<i>Dichelostemma captatium</i> ssp. <i>captatium</i>	2	0	2	0
<i>Eriogonum cinereum</i>	4	0	3	1
<i>Hazardia squarrosa</i>	1	1	0	0
<i>Leymus condensatus</i>	26	0	17	9
<i>Malacothamnus fasciculatus</i> var. <i>fasciculatus</i>	20	7	9	4
<i>Malosma laurina</i>	24	1	4	19
<i>Marrubium vulgare</i> *	3	3	0	0
<i>Nassella pulchra</i> or <i>Nassella lepida</i>	108	10	59	32
Non-native grass: <i>Avena</i> sp*, <i>Bromus</i> sp*, <i>Erharta</i> sp*, or <i>Phalaris</i> sp*	284	182	90	12
<i>Pseudognaphalium californicum</i>	3	2	1	0
<i>Phacelia ramosissima</i>	4	0	2	2
<i>Quercus agrifolia</i> var. <i>agrifolia</i>	1	1	0	0
<i>Quercus lobata</i>	1	1	0	0
<i>Rhamnus ilicifolia</i>	1	1	0	0
<i>Salvia leucophylla</i>	33	3	13	17
<i>Sambucus nigra</i>	2	0	1	1
<i>Acemispom glaber</i> [formerly <i>Lotus scoparius</i>]	40	7	31	2
<i>Toxicodendron diversilobum</i>	2	0	0	2
<i>Trichostema lanceolatum</i>	4	0	1	3
Unknown	9	9	0	0
No Change	247	39	105	103

goodness-of-fit test, using the uniform probability distribution for the expected probabilities.

Sample size selection was governed by the availability of suitable sites, so we ran scenarios to learn if we had a sample large enough to minimize the probability of a Type II error (β). Not knowing ahead of time if there would be an effect of *B. pilularis* on the re-establishment of native species or what magnitude such an effect might have, we estimated the statistical power of our sample sizes, using G*Power (Faul et al. 2007), hoping to achieve at least a 0.80 probability of avoiding a false negative finding ($1 - \beta$). Conservatively assuming the effect (w) would be “weak” in Cohen’s usage (effect size, w , of 0.20), even the smallest of the three tables attained power in excess of 0.99 (Cohen 1992). Post-hoc power achieved and effect size are noted for each result in addition to statistical significance (P-value).

RESULTS

The surveys encountered 1218 individual plants over the 1090 data collection points (due to problems of access, 21 of the original 1111 data locations were not considered for analysis). There were 30 designations assigned that included 23 native species, five non-native species, no change, or unknown (Table 1, Fig. 6). As expected, *B. pilularis*, non-native grasses,

and no change were the most commonly encountered designations.

Age classification revealed spatial variations across all transects (Fig. 6). While photo analysis revealed shrub stand expansion occurred in general since 1976, each specific transect location contained varying proportions of age classes.

Separating each summarized age class into native and non-native vegetation types revealed distinct patterns. Vegetation in age class A showed non-native species were found in 95 percent of the sampling data points, as opposed to 38 percent for native species. Of the 436 plants encountered in class A, 29% were native, while 71% were non-native. Vegetation in age class B showed a marked increase in native species while non-natives declined sharply (Fig. 7): 74% of sampling data points contained native species, while only 39% of them contained non-natives. Of the 492 plants encountered in class B, 65% were native and 35% non-native. Vegetation in age class C showed a moderate relative increase in natives with 80% of sampling points containing natives, while non-natives again declined sharply, with only 10% of sampling points containing non-natives (Table 2). Interestingly, the number of plants subsides in these oldest classes to 290. Of these 90% were natives and 10% were non-natives. The observed counts of native and non-native plants

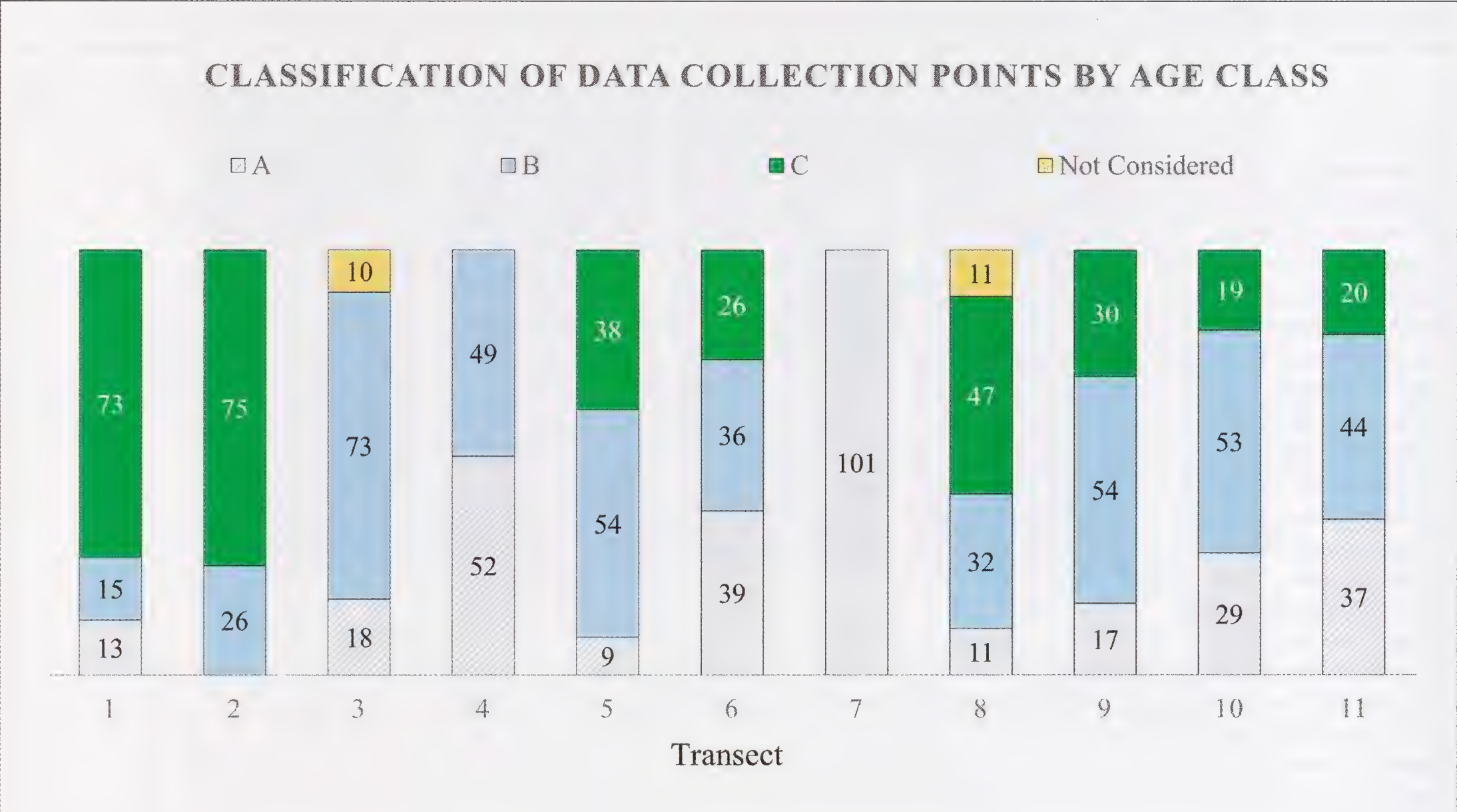


FIG. 6. Classification of data collection points and quantities within each respective age class. A < 12 yr in age; 12 < B < 23 yr in age and C > 23 yr in age.

were significantly different among age classes ($\chi^2 = 290.103$, $df = 2$, $P < 0.001$). The effect size is moderate (0.485), so the sample size ($n = 1218$) was sufficient to achieve a power of 0.999.

When examining *B. pilularis* abundance within each age classification for signs of facilitation of other CSS species, results show a non-monotonic increase in native species (Table 3). Of the 125 native plants encountered in class A, 30% were *B. pilularis*. The number of natives increased in class B to 322, as did the number of *B. pilularis*. Coyote brush, however, comprised only 26% of natives in this age class. In class C, the number of natives decreased to 262, but the 92 *B. pilularis* made up 35% of them.



FIG. 7. Age class A (foreground) transitioning to age class B (background) (Photograph by Sean Brennan).

However, these changes in the percentage of *B. pilularis* are roughly similar across the three age classes. *Stipa pulchra* Hitchc. (Poaceae), and *S. lepida* Hitchc. (Poaceae) were among the native species whose numbers increased non-monotonically with the age of the *B. pilularis* cover. These native bunchgrasses increased from 10 in class A to 59 in class B, thereafter subsiding to 39 under class C. This increase in *Stipa* species accompanies a strong decline in non-native grasses, from 182 in class A, through 90 in class B, down to only 12 in class C.

A Chi-square analysis of *B. pilularis* frequency relative to all other native species frequency revealed no significant differences ($\chi^2 = 5.196$, $df = 2$, $P = 0.074$, $1 - \beta = 0.509$) with almost no shift in the balance between *B. pilularis* and the other native species over the three time classes ($w = 0.086$).

When comparing summations of *B. pilularis* within each age class to the presence of non-native vegetation, however, results show a substantial negative association. As age classes became more populated with *B. pilularis* over time, non-native vegetation noticeably declined. A Chi-square analysis

TABLE 2. FREQUENCY OF NATIVE AND NON-NATIVE SPECIES BY AGE CLASS (A < 12; 12 < B < 23; C < 23 YR OF AGE).

Age Class	Native	Native %	Non-native	Non-native %
A	125	38	311	95
B	322	74	170	39
C	262	80	28	9

TABLE 3. FREQUENCY OF *B. PILULARIS* AND ALL OTHER NATIVE SPECIES BY AGE CLASS (A < 12; 12 < B < 23; C < 23 YR OF AGE).

Age class	Native species	Coyote brush	%	All other natives	%
A	125	38	30	87	70
B	322	85	26	237	74
C	262	92	35	170	65
Total	709	215	30	494	70

reveals a small, but significant, inverse association between the frequencies of *B. pilularis* and non-native species ($\chi^2 = 187.558$, $df = 2$, $P < 0.001$, $1 - \beta = 0.997$, $w = 0.136$).

Finally, shrub canopy extents most often featured the attribute of no change. No change values increased with shrub stand age as the vegetation approached a stable, native-dominated equilibrium (i.e., type-conversion back from exotic-dominated grassland to a native-dominated scrub). Most noticeable is the 83% increase from age class A to age class B, followed by a 41% increase from age class B to age class C ($\chi^2 = 31.941$, $df = 2$, $P < 0.001$, $w = 0.368$, $1 - \beta = 0.961$).

DISCUSSION

Our analyses indicate that expanding stands of *B. pilularis* support increased native plant cover. Following initial shrub invasion (age class A < 12 yr), a modest diversity of native species was found to exist. Then, following expansion or establishment of additional *B. pilularis* (age class 12 < B < 23 yr), non-native species declined precipitously while native shrubs, such as *Artemisia californica*, *Malosma laurina*, *Salvia leucophylla*, and native grasses, such as *Elymus condensatus*, *Stipa pulchra*, and *Stipa lepida*, increased. Finally, after canopy closure (age class C > 23 yr), analysis showed native species diversity declined slightly while non-native species almost disappeared.

Results thus suggest that *B. pilularis* invasion of exotic grasslands can facilitate the reestablishment of other CSS species, as well as native bunch grasses, over time. We found a relatively high level of native species richness distributed across all eleven transects. Natives accounted for 12 of the 29 species identified. These results show *B. pilularis* stands are associated with native species richness. This diversity included a substantial native grass population as well as several species of shrubs, trees, and surprisingly the endangered *Astragalus brauntonii* Parish, where 11 of 15 individuals encountered occurred in stands older than 23 yr of age (age class C). Native grasses accounted for 108 of the 1218 individual plants encountered (9% of all vegetation). Non-native grasses were more abundant overall than native grasses. There were 284 non-native grass individuals encountered (23% of all vegetation), considerably more than native grasses. The native grasses,

however, increased in class B, while non-native grasses declined steadily from class A to class C.

Our results support those of Zavaleta and Kettley (2006) who also found that over time, native species colonized the area beneath the *B. pilularis* canopy and that exotic cover gradually declined with time in the San Francisco Bay Area. However, in northern Californian cases, where precipitation is notably higher than in the Santa Monica Mountains, *B. pilularis* invasion of grasslands led to a shift to woodland cover with low native species richness (Hobbs and Mooney 1986; Williams et al. 1987; Russell and McBride 2003; Zavaleta and Kettley 2006) while, in our southern California context, coyote brush invasion increased native shrub and grass species richness over time, a phenomenon which has not been documented in prior studies on coyote brush.

The frequency of native and non-native species within each age class revealed stark differences. Portions of shrub stands less than 12 yr of age contained the highest percentage of non-native species, while also having the lowest percentage of native species. Nearly every data collection point (95%) in age class A contained a non-native plant, while far fewer (38%) contained a native plant. This suggests that while the vegetation composition in this age class consisted primarily of non-native annuals, native species can co-exist under specific circumstances.

The high percentage of non-native species in age class A may reflect increased stress or intense competition. When considering portions of transects that are at least 12 yr of age but less than 23 (class B), however, a reversal of vegetation dominance transpires. The percentage of data collection points having a native plant nearly doubles (from 39% to 74%). Surprisingly, native grasses increased despite increased shrub cover—only 10 individuals were found in age class A (3% of data points in this class), followed by 59 in age class B (14% of data).

When analyzing portions of transects at least 23 yr of age, the data suggest that an ecological threshold has been breached, and that a transition to a new state of shrubland is occurring. This is supported by the fact that native species were found in 80% of all age class C data points, while non-native grasses were found in just 9%. In addition, native grasses remained relatively high with 39 individuals identified in age class C (12% of all age class C data collection points). The vegetation composition in age class C indicates that a transition to a CSS/grassland mix is underway. This finding is also consistent with Zavaleta and Kettley (2006), who used a similar methodology to document the role of coyote brush in facilitating the conversion of exotic grasslands to woodlands in the San Francisco Bay Area.

As documented, the rapid establishment of coyote brush canopy can inhibit some species, especially annual grasses, from establishing. This canopy effect was measured in the field by the attribute of no

change. While the majority of no change values followed *B. pilularis* individuals, other less encountered species in the no change category included *Artemisia californica* and *Malosma laurina*. Considering that *B. pilularis* stands were singled out for this study, an expectation would follow that over time, increasing shrub canopies would shade out lower stature vegetation regardless of being native or non-native. In addition, nearly monospecific stands of *B. pilularis* or other CSS shrub species would come to dominate the landscape over time. However, when analyzing the frequency of *B. pilularis* with all other native species, there was minimal effect with stand age (see Table #). Although *B. pilularis* stands appear as a monoculture when viewed from the stand edge or using aerial imagery (Figs. 1 and 5), when observed *in situ* or from within a shrub stand, a broad mix of native species becomes apparent.

Importantly, the process of facilitation can be observed where portions of the shrub canopy are no longer contiguous. As noted previously, at approximately nine yr of age, most *B. pilularis* individuals begin the process of senescence. These relatively older shrubs begin to break down physically, creating gaps in the canopy that permit other species to germinate. This continual breakdown of *B. pilularis* individuals over time may partially explain the non-monotonic increase of native vegetation across all age classes, from 125 through 322 to 262 in classes A, B, and C, respectively. *Baccharis pilularis*, meanwhile, is a relatively static element of the vegetation mix throughout, its share of the native species varying only from 30% to 26% to 35% in those age classes.

The classification of transects into age classes revealed considerable variations in individual extents. The fact that all age classes did not appear uniform across the eleven transects illustrates the diverse trajectories of recovery that shrub stands are experiencing in the Santa Monica Mountains. These variations may be reflective of past land use issues, such as the type and severity of disturbance and time since disturbance or variations in edaphic conditions or a combination. Research has shown that following the release from grazing, CSS shrub recovery in the Santa Monica Mountain region was most rapid in locations not previously disturbed mechanically, which tend to be located on steeper, or rockier, slopes (Laris et al. 2017). Many areas where intensive mechanical disturbance occurred experience very slow shrub recovery and have remained covered by non-native annual grasslands for decades (Engelberg et al. 2013). This study finds that the presence of coyote brush tends to counteract the impact of past mechanical disturbances and to facilitate native plant recovery.

Conclusion

Large areas of southern California remain under exotic annual grass cover, while areas under native

vegetation continue to decline or remain threatened. Although there have been, and are, numerous efforts to restore these areas and replace the exotics with native species, there remain key questions as to the nature of the original plant cover. Notions of the native plant cover of the foothills and valleys of southern California have been heavily influenced by Clements' "bunch-grass hypothesis," which assumes that areas currently dominated by exotic annual grasses were at one point covered in native bunch grasses (Clements 1934). The influence of this theory is quite apparent for the La Jolla Valley, a focus of this study, which was established as a preserve in 1972 to encourage native grassland recovery (Goode 1981; Gale 1983). As noted, however, recent evidence casts considerable doubt on the notion that bunch grasses covered large areas of southern California (Hamilton 1997; Schiffman 2005; Minnich 2008). Nonetheless, the impression that the exotic grasslands must have been native "grasslands" in the past is a difficult one to shake, especially given the fact that few areas of native grasses remain in the southern part of the state (Minnich 2008). As such, it is important to consider the findings of this study within the broader context of these theories and ideas.

This study finds that coyote brush is currently advancing into well-established exotic grasslands in the Santa Monica Mountains. Although in some areas the shrub appears to form a contiguous monotypic stand, at least in the short term, the long term results suggest that coyote brush gradually gives way to, or facilitates, the establishment of a mix of native plants. In the southern Californian foothills and valleys studied here, young coyote brush stands contain a high percentage of exotic species, primarily grasses, and few native ones. Conversely, mature stands of coyote brush support a wide variety of both native shrubs and grasses beneath the canopy, while exotics are reduced to less than 10% of the cover.

While it is possible that the species recovering beneath the coyote brush canopy reflect what existed pre-disturbance (at least pre-European contact), we are cautious when interpreting the results in this manner. First, our previous research suggests that disturbance intensity plays a critical role in determining current vegetation cover in southern Californian coastal valleys and hillsides and that the intensity of disturbance was also a function of various factors including elevation, slope, and soil rockiness (Engelberg et al. 2013; Laris et al. 2017). Therefore, although the results presented here indicate that coyote brush stand age is a critical determinant of native species presence, the stands we studied were advancing downhill and thus the findings may be subtly influenced by other factors, such as soil type or disturbance regime history. When considering La Jolla Valley, for example, while it is quite clear that coyote brush has rapidly advanced into areas on slopes where previously mechanical disturbance has been used to remove shrubs, it is not



FIG. 8. A mix of CSS shrubs recovering three yr after the 2013 Spring fire on a site formerly dominated by coyote brush. Note the large burned stems of dead coyote brush shrubs, lack of dense exotic grass cover and presence of bare soil (Photograph by Paul Laris).

clear whether coyote brush will continue to advance onto the valley floor which is still dominated by grasses. We do not doubt the facilitating role played by coyote brush; rather, we suggest caution when interpreting the results suggesting that CSS shrubs will continue to be dominant in the understory as the coyote brush advances into lower elevations with more gradual slopes and deeper soils, areas where, it has been argued by some, that native grasses and forbs would have been the dominant cover (Wells 1962; Callaway and Davis 1993; Keeley 2005). It should be noted, however, that Zavaleta and Kettley (2006) documented that coyote brush facilitated the conversion of previously disturbed level valley floors from grassland to woodland at Stanford University's Jasper Ridge Biological Preserve in San Mateo County, northern California.

Thus, whether coyote brush is advancing into areas that were once dominated by native shrubs or areas that were historically covered by grasses and forbs is difficult to determine from our results at this point in time at this site. As has been argued elsewhere, shrubland/grassland boundaries, disturbance regimes, and edaphic conditions often overlap, making it difficult to determine original vegetation cover in coastal California (Wells 1962; Laris et al. 2017). Moreover, it may well be the case that cycles of disturbance and recovery (whether human- or climate-induced) have long caused shifting of grassland/shrubland boundaries in California. Coyote brush may simply be an opportunistic species that has caused a temporary landscape cover shift that may eventually lead to yet more change over time. Indeed, if the current post-fire recovery pattern in the la Jolla Valley, for example is indicative of more general processes of change, coyote brush is not recovering on the upper-slopes (although it is on the lower ones), suggesting that the specific set of conditions that led to its invasion have now changed.

The vegetation on the upper slopes has shifted to a mix of predominantly native shrubs after the 2013 fire (Fig. 8). The current vegetation pattern might be explained by the fact that coyote brush is known to be fire-sensitive and able to resprout from light canopy fire but not from more intense fires that burn the base of the plant (Steinberg 2002) or that coyote brush is most successful on moist soils.

In closing, the results of this study might prove useful for restoration biologists who seek to speed-up the recovery of native species in areas long dominated by exotic grasses in southern California coastal areas. Our findings support the "*Baccharis* hypothesis" put forth by DeSimone and Zedler (2001) that coyote brush can facilitate recovery of a more diverse mixture of species on the landscape by removing or damping the competition from exotic grasses. Our results suggest that it is probable that establishing a stand or belt of coyote brush at the up-wind edge of an area of exotic grasses might be effective at facilitating the recovery of a wide variety of CSS and native grass species over time, at least in some settings, especially in southern California where annual precipitation is low and variable. Although the process of recovery may take up to 20 yr or more, many areas dominated by exotic grasses today have remained devoid of native shrubs or grasses for a century or more; as such, coyote brush may well be effective at speeding the restoration process.

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NOVELTIES IN *MUSINEON* (APIACEAE) AND *ORTHOCARPUS* (OROBANCHACEAE)
IN THE NORTHERN WASATCH MOUNTAINS OF UTAH AND IDAHO

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ABSTRACT

Two novelties from the Bear River Range of the northern Wasatch Mountains of Utah and Idaho are *Musineon naomiensis* L. M. Shultz & F. J. Smith, sp. nov. (Apiaceae) and *Orthocarpus holmgreniorum* (T.I. Chuang & Heckard) L. M. Shultz & F. J. Smith, comb. et. stat. nov. (Orobanchaceae). With these additions, the Bear River Range harbors 13 endemic plant species, most of which are edaphically restricted to dolomitic rocks in or near Logan Canyon. Most of the 260+ species of the 2600+ native species on the Utah state list of rare plants are in the warm-desert portion of the state. The montane endemics enumerated here demonstrate the unique habitats of the mountains of northern Utah and distinguish the Bear River Range as a hotspot of rarity.

Key Words: Bear River Range, edaphic endemism, glacial refugia, *Musineon* (Apiaceae), northern Wasatch Mountains, *Orthocarpus* (Orobanchaceae), rare plants, Utah.

Utah is known for its high proportion of narrowly restricted species of flowering plants. More than 10% of its approximately 2700 native species are endemic to the state (Albee et al. 1988; Welsh et al. 2008), making the area one of the richest areas of endemism in North America (Stein et al. 2000). Of the 260 endemic species listed as rare and restricted to unique edaphic substrates (Welsh et al. 1978; Stohlgren et al. 2005; Fertig 2012), the majority are restricted to low elevation habitats with low species diversity (Shultz 1993).

Although mountain endemics are uncommon in Utah (Shultz 1993), the Bear River Range of the northern Wasatch Mountains harbors 13 endemics, eight of which are known only from Logan Canyon. The high concentration of endemics signals the Logan Canyon area as an unusual hotspot of rarity in Utah. The two species described here – one with a new status, the other as a novel species – add to a growing list of endemic species in northern Utah.

Notable among these species is the Maguire primrose (*Primula maguirei* L. O. Williams), the only one of the named local endemics that is currently protected with threatened status (US Fish and Wildlife Service 1985). It shows strong genetic differentiation among closely related species in the *Primula cusickiana* (A. Gray) A. Gray complex, as well as surprisingly high levels of heterogeneity within its small geographic range (Wolf and Sinclair 1997; Bjerregaard and Wolf 2008; Davidson et al. 2014). We can infer from these genetic studies as well as the widespread geographic range of *P. maguirei* (Kass and Welsh 1985; Kelso 1991; Holmgren and Kelso 2001) that it is a glacial relict.

The rare plants of Logan Canyon provide an opportunity to study evolutionary forces leading to

relictual endemism, as well as neo-endemism, patterns that can be either anagenetic or cladogenetic (Takayama et al. 2015). The two species described here appear to be closely related species, both nested within species complexes inhabiting mountain ranges of the Intermountain region, which stretches from the Sierra Nevada of California to the Colorado Plateau of Arizona, Colorado, New Mexico, and Utah.

TAXONOMIC TREATMENT

Musineon naomiensis L. M. Shultz & F. J. Smith, sp. nov. (Fig. 1).— TYPE: USA, UTAH, Cache Co., Naomi Peak, Mt. Naomi Wilderness area, Bear River Range of the Wasatch Mountains, 41.9114 N, 111.6686 W, in shaded sites, crevices of limestone, SSE exposure, with *Clematis*, *Penstemon*, *Mertensia*, *Leucopoa kingii*, 2 Jul 2013, Frank J. Smith 4225, with Mike Jablonski and Jane Catlin (Holotype: UTC; isotypes: NY, JEPS).

Diagnosis. The narrowly endemic *Musineon naomiensis* has white petals 2–3.5 mm long marked with a dark red center, a compact growth form, bractlets 2–4 mm long, glabrous fruit, and a subalpine distribution (Fig. 2A). The closely related and more common *Musineon lineare* (Rydb.) Mathias has yellow petals 4–8 mm long, bractlets 4–7 mm long, scaberulous fruit, a sprawling growth form, and mid to upper montane habit (Figure 2B). In the few sites where the two species are sympatric, they remain morphologically distinct with no evidence of hybridization.

Plants caespitose perennial from a woody base, forming clumps 3–4 cm in diameter, 3–5 cm high,



FIG. 1. *Musineon naomiensis* L. M. Shultz and F. J. Smith, sp. nov. (A) Habit. (B) Fruit. Illustration by Lara Call Gastinger.

with persistent leaf bases; acaulescent, faintly aromatic. **Roots** thick taproot that is usually branched. **Leaves** pinnately compound with 5–7 leaflets, 2–5 (–7) X 1–2 cm, rarely twice-pinnate, broadly lanceolate in outline, glabrous, dark green on both surfaces, leaflets linear-oblongate, 3–5 (–10) X 1.0–1.5 mm, with revolute margins, apical leaflets longer than the laterals; petioles 1–3 cm long with conspicuous papery sheaths. **Inflorescence** a compound umbel, compact, 5–8 (–10) mm

diameter, terminal on a naked scape immersed in, or barely exceeding the leaves; scape 4–7 cm (–14) cm long. **Bracts** linear-elliptic (broadest in the middle), 2–4 mm long, scarious-margined; bracteoles absent. **Calyx** teeth to 0.5 mm long. **Petals** white with a dark red mid-section, 2.0–3.5 X 0.9–1.0 mm. **Styles** 0.8–1.0 mm long. **Anthers** white, ca. 0.5 mm long; stylopodium wanting; carpophore entire or obscurely bifid. **Fruit** schizocarp subterete, 2.0–4.0 X 1.5 mm, glabrous, ribs white, conspicuous.

Paratypes: USA, IDAHO, **Bear Lake Co.:** 0.25 mi S of Bloomington Lake, Bear River Range, ca. 11 air miles NW of St. Charles, Cache National Forest, T15S, R42E, SW1/4 Sec. 4; elev. 9000 ft; N to NW aspect, on limestone, with *Pinus flexilis*, *Pachistima myrsinites*, *Pellaea breweri*. 19 Jul 1990. M. Mancuso 286 (NY, ID); cirque headwall above Bloomington Lake, Bear River Range, ca. 9 miles WNW of St. Charles, T15S, R42E, SW1/4 Sec. 5, elev. 8800 ft, on Laketown dolomite; moist ledges, cliff faces, with *Aquilegia coerulea*, *Poa nervosa*, *Abies lasiocarpa*, 18 July 1990, R. Moseley and M. Mancuso 1793 (ID). UTAH, **Box Elder Co.:** Wellsville Mountains, Wellsville cone, T11N, R2W, Sec 36, elev. 8800 ft, 23 Jul 1991 (long-leaved form), F. J. Smith 3501 (UTC); SE of Box Elder Peak, T10N, R2W, S1, elev. 9280 ft, 23 Jul 1991 (scapes to 14 cm), F. J. Smith 3506 (BRY). **Cache Co.:** south slopes above White Pine Lake, elev. 8700 ft, 17 Jul 1936, Bassett Maguire 14098 et al. (UTC); vicinity of Mt. Naomi, 5 July 1937, R.S. Snell s.n. (BRY, UTC); east slope of Mt. Naomi, elev. 9300 ft, 18 Aug 1938, B. Maguire 16187 (BRY, UTC); east of Naomi Peak summit, crevices of rocks,



FIG. 2. Photos showing the white-flowered *Musineon naomiensis* (A) and yellow-flowered *Musineon lineare* (B). Photographs by Frank J. Smith.

30 Jul 1944, *A.H. Holmgren* 3595 (UTC); infrequent on rock faces along ridge to south slope of Mt. Naomi, 23 Jul 1953, *S. Tillett* 276 (NY, UTC; bare limestone outcrop near summit of Mt. Naomi, elev. 9100 ft, 25 Jun 1981, *L. Shultz* 5401, with J. Shultz, C. Loveland, and D. Thomas (UTC); summit of Mt. Naomi, 3.5 mi above Tony Grove Lake, 24 Jun 1987 (voucher for chromosome count sent to R. Hartman), *L. M. Shultz* 10475 with J. Shultz, D. and J. Cade (RM, UTC); Bear River Range, Smithfield Canyon, 2.8 mi up from campground, T13N, R2E, NWSW1/4 Sec. 3, elev. 8200 ft, limestone cliff, with *Petrophytum*, 17 May 1989, *F. J. Smith* 3003 (BRY) [*bracts to 6 mm]; south fork of High Creek, Bear River Range, T14N, R2E, NENE1/4 Sec. 26; elev. 7640 ft 29 May 1989, *F. J. Smith* 3023 (BRY); south fork of High Creek, Bear River Range, T14N, R2E, SWSW1/4 Sec. 11; 29 May 1989, *F. J. Smith* 3027 (BRY); Naomi Peak Wilderness area, NW slope of ridge (between) Smithfield and Cottonwood Canyons, T13N, R2E, SWSE1/4 Sec. 11, elev. 8800 ft; crevices and soils in dolomite outcrops, spruce-fir community, 20 July 1989, *M. A. (Ben) Franklin* 6848 (BRY); NW ridge of Cherry Peak, Naomi Peak Wilderness Area, T14N, R2E, NWSE1/4 Sec. 26, elev. 9120 ft, 21 July 1989, *M. A. (Ben) Franklin* 6850 (BRY) [*annotated by Fengjie Sun as *M. lineare* on 6 Jan 2001]; exposed limestone near Mt. Naomi, in crevices, 41°54'71.0"N, 111°40'107"W, elev. 9452 ft, 9 Jul 2008 (in bud), *L. M. Shultz* 20658 (UTC); below Mt. Naomi, 41°54'40.7"N, 111°40'05.9"W, elev. 9404 ft, 9 Jul 2008 (in bud), *L. M. Shultz* 20656 (UTC).

DISTRIBUTION, HABITAT, AND ABUNDANCE

The Mt. Naomi musineon is both rare and edaphically restricted. It is found on dolomitic limestone of the Silurian Laketown formation and its habitat is restricted to high elevation rock crevices. It occurs both in the Bear River Range (Utah and southern Idaho) and Wellsville Range (Utah) of the northern Wasatch Mountains. Our searches of similar habitats in Idaho, Utah, and Wyoming have failed to extend its range. Surveys by botanists doing rare plant surveys (Shultz and Shultz 1979; Moseley and Mancuso 1990) support our conclusion that the species is indeed isolated and extremely rare. The documented populations grow on exposed rock outcrops within subalpine communities dominated by *Picea engelmannii* Engelm. (Engelmann spruce), *Abies lasiocarpa* (Hook.) Nutt. (subalpine fir), and *Pinus flexilis* James (limber pine). Associated herbaceous species include *Heuchera rubescens* Torr., *Pellaea breweri* D. C. Eat., *Draba maguirei* C. L. Hitchc. var. *maguirei*, *Erigeron eatonii* A. Gray, *Ranunculus adoneus* A. Gray, *Aquilegia caerulea* James, *Mertensia ciliata* (Torr.) G. Don, *Clematis occidentalis* (Hornem.) DC., and *Penstemon leonardii* Rydb.

ETYMOLOGY

We have chosen the species name for the location of the largest known population and type locality within the Mt. Naomi Wilderness Area of the Bear River Range of Utah and Idaho and propose using Mt. Naomi musineon as the common name.

TAXONOMIC AFFINITIES

Considered by earlier authors as conspecific with the yellow-flowered *Musineon lineare* (Rydb.) Mathias, *M. naomiensis* is distinctive both morphologically and ecologically (Fig. 2). *Musineon* is a phylogenetically problematic genus whose relationship within Apiaceae is unclear (Downie et al. 2002; Sun et al. 2004; Sun and Downie 2004, 2010). Originally described as *Daucophyllum lineare* Rydb., *M. lineare* also shares morphological similarities with some species of *Cymopterus*, *Orogenia*, and *Harboulia*.

TAXONOMIC TREATMENT

Orthocarpus holmgreniorum (T. I. Chuang & Heckard) L. M. Shultz & F. J. Smith, comb. et stat. nov., based on *Orthocarpus tolmiei* Hook. & Arn. subsp. *holmgreniorum* T. I. Chuang and Heckard, Systematic Botany 17(4): 565-. 1992. (Fig. 3) — TYPE: USA, UTAH, Cache Co., along Tony Grove Lake Road, Bear River Range, Wasatch Mountains, ca. 2 mi (3.2 km) from route 89 in Logan Canyon, above and northwest of the Utah State Forestry Field Station, 6750 ft (2057 m), 41.876447 N, 111.573029 W, 2 Aug 1984, *Heckard* 6169 (holotype: JEPS; isotypes: F, GH, MO, NY, US, UTC), *n* = 14. **Diagnosis.** Holmgren owl clover is distinguished by its pinkish purple flowers, narrowly-branched inflorescence, glandular-pubescent galea, densely glandular calyx, and dark-green leaves (Fig. 3A). In contrast, *O. tolmiei* has yellow flowers, a widely branched inflorescence, glabrous galea, sparsely glandular calyx, and light-green leaves (Fig. 3B). When Chuang and Heckard described *Orthocarpus tolmiei* subsp. *holmgreniorum*, so little was known of its distribution and ecology that they hesitated to describe it as a distinct species even though that was their initial intent (L. Heckard, pers. comm.).

Paratypes: USA, IDAHO, **Caribou Co.**, Elk Valley, Caribou National Forest, *Cronmiller* 348 (USFS). **Franklin Co.**: Mill Canyon, USFS Grazing Reconnaissance 265 (USFS). UTAH, **Box Elder Co.**: Willard Basin, *Cottam et al.* 14924 (ARIZ, RSA). **Cache Co.**: Tony Grove, 4 Jul 1932, *F. B. Wann* s.n. (UTC); vicinity USU Forestry Camp, 24 Jul 1937, *R. S. Snell* s.n. (UTC); West Hodges Pasture, 11 Jul 1935, *H. B. Passey* 68 (UTC); Hardware Ranch, 5300–6300 ft, 23 Jul 1972, *M. A. Bayoumi* s.n. (UTC); Bear River Range, Logan Canyon, Tony Grove Lake road, 2.7 km (1.7 miles) above US hwy 89; T13N, R3E, S11, 2010 m (6600 ft) elev., locally common



FIG. 3. Photos comparing the pink-flowered *Orthocarpus holmgreniorum* (A) and the yellow-flowered *Orthocarpus tolmei* var. *tolmei* (B). Photographs by Wayne Padgett.

with low sagebrush, 11 Aug 1984, *N. H. Holmgren and P. K. Holmgren* 10673. (NY, UTC); along Tony Grove Lake road, common in dry, hard soil openings, elev. 6750 ft 14 Aug 1984, *L. R. Heckard* 6211. (JEPS, UTC); road to Tony Grove, 41°52'40"N, 111°29'3.8"W, in shallow clay soil with *Artemisia arbuscula*, *Elymus spicatus*, *Madia glomerata*, *Ceanothus velutinus*, 23 Aug 1985, *L. M. Shultz* 8815 et al. (UTC, JEPS, NY); 1.5 km E of Logan Canyon and 1.4 km N of right hand fork road, dry meadow with *Wyethia amplexicaule*, *Artemisia tridentata*, *Rosa woodsii*, elev. 2025 m; UTM 447986E, 4626681N, 9 Jul 2003, *E. Johnson* 08-03-E. (UTC); 1 km E of Logan Canyon and 1 km N of right hand Fork, UTM 447461E, 4626691N, elev. 2104 m, 9 Jul 2003, *E. Johnson* 08-01-B (UTC); road to Tony Grove 41°52'37"N, 111°33'43"W, 2002 m elev., 29 Sep 2005 [in fruit], *L. M. Shultz* 20221. (UTC). **Morgan Co.:** approx. 5 mi N of Mountain Green, *Albee* 3236 (UT). **Rich Co.:** Swan Peak Road, elev. 8200 ft, T14N, R4E, Sec. 10 [41°58'15.3"N, 111°29'3.8"W], limestone outcrop, steep hillside with snowberry, sagebrush, mountain mahogany, 20 Aug 1983, *K. Thorne* 2977 (BRY, UTC). **Wasatch Co.:** Strawberry Divide, 31 Jul 1955, *H. E. Ahles* 9765 (UTC). **Weber Co.:** Mt. Ogden, Snow Basin ski area, *S. Clark* 2167 (BRY, UTC).

ETYMOLOGY

We retain the name *holmgreniorum* in honor of Noel Herman Holmgren and Patricia Kern Holmgren, primary authors of the Intermountain Flora (Cronquist et al. 1973+) and extraordinary botanical explorers of western North America.

DISTRIBUTION AND HABITAT

Orthocarpus tolmei Hook. & Arn. subsp. *holmgreniorum* T. I. Chuang & Heckard (Holmgren owl clover) was first described as a purple-flowered, annual, unbranched plant with a narrow inflorescence, occurring in the Wasatch Mountains (Chuang and Heckard 1992; Fig.3A). The species is found in the Wasatch Mountains, from areas north of Salt Lake City to southern Idaho. It grows on shallow, rocky, clay soils dominated by *Artemisia arbuscula* Nutt. subsp. *thermopola* Beetle (low sagebrush), *Madia glomerata* Hook. (tar weed), and associated grasses. It is found near populations of the more common, yellow-flowered *O. tolmei* Hook. & Arn. subsp. *tolmei* (Yellow owl clover; Figure 3B) which typically grows in soils with higher organic matter and in association with *Artemisia tridentata* Nutt. subsp. *vaseyana* (Rydb.) Beetle (mountain big sagebrush). In the few places where we have seen the pink-flowered and yellow-flowered plants growing together, we have seen no evidence of hybridization. The lack of apparent introgression and distinctive morphologies has led us to treat the purple-flowered form (sometimes mistaken for *O. purpureo-albus* A. Gray) as a distinct species.

DISCUSSION

The Mt. Naomi musineon and Holmgren owl clover make a total of thirteen rare and endemic plant species found in Logan Canyon, eight of which are known only here. These are *Erigeron cronquistii* Maguire, *Draba maguirei* C. L. Hitchcock, *Musineon lineare* (Rydb.) Mathias, *Musineon naomiensis* L. M. Shultz & F. J. Smith; *Orthocarpus holmgreniorum* (T.

I. Chuang & Heckard) L. M. Shultz & F. J. Smith; *Penstemon compactus* (Keck) Crosswhite, *Primula maguirei* L. O. Williams, and *Viola frank-smithii* N. Holmgren. The five other endemic species found in Logan Canyon as well as other places in the northern Wasatch Mountains are *Arabis lasiocarpa* Rollins, *Eriogonum loganum* A. Nels., *Ericameria obovata* (Rydb.) G. L. Nesom, *Lesquerella multiceps* Maguire, and *Tonestus kingii* (D. C. Eat) G. L. Nesom. Detailed descriptions of all thirteen species can be found in the *Illustrated Flora of the Intermountain Region* (Cronquist et al. 1973+) and *Flora of North America North of Mexico* (Flora of North America Editorial Committee, 1993+). As evolutionary theory and studies of these narrow endemics progress, we hope to arrive at a better understanding of the origins and relationships of these species as well as patterns of rarity.

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We are grateful for the tireless work of botanists who search for endemics in Utah. We thank Michael Piep of the Intermountain Herbarium (UTC) for obtaining loans, and the curators of herbaria who sent specimens from BRY, JEPS, ID, MO, NY, RM, RSA, and UT. Noel Holmgren, Steven Downie, Bill Weber, and Paul Wolf reviewed an early draft and provided helpful commentary. The Ecology Center of Utah State University provided funding for the illustration by Lara Call Gastinger, adding to her artistic portrayal of Logan Canyon endemics for the Utah Native Plant Society.

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—SUSAN J. MAZER, President, California Botanical Society, Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, 93106, mazer@lifesci.ucsb.edu.

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NOTEWORTHY COLLECTION

CALIFORNIA

Eremothera nevadensis (Kellogg) W.L. Wagner & Hoch [= *Camissonia nevadensis* (Kellogg) P.H. Raven] (ONAGRACEAE). Mono County, Bodie Hills, 4.0 km past forest boundary on road to Masonic Mountain, T6N, R26E, Sec. 9, 2134 m, (estimated coordinates of 38.375396°, -119.117734° provided by Matt Lavin), a tap-rooted annual, petals white, with *Pinus monophylla* Torr. & Frém., *Juniperus osteosperma* (Torr.) Little, and *Artemisia tridentata* Nutt., 10 June 1980, Matt Lavin SW31 (RENO, two sheets, barcodes 1803 and 1814).

Previous knowledge. *Eremothera nevadensis* was thought to be endemic to Nevada (Cronquist et al. 1997). It is currently known in Nevada from Carson City Consolidated Municipality (formerly Ormsby County) and from Churchill, Douglas, Lyon, Pershing, Storey, and Washoe counties.

Significance. This is the first report for California. It is not listed for California in Cronquist et al. (1997), or Wagner (1993, 2012). The closest known location is about 14.5 kilometers ENE in Lyon County, Nevada. The senior author is working on an annotated checklist for the flora of Mono County, California. In preparation for a visit to the RENO herbarium she searched the RENO database (hosted by the Intermountain Region Herbarium Network in

collaboration with SEINet) for specimens from Mono County. The search returned over 2300 specimens, including *Eremothera nevadensis*, which was a first record for Mono County, and for California. The two *Eremothera nevadensis* specimens cited above have been in the RENO herbarium for over 35 yr and in the database since October 2014, but their significance was not previously realized.

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WATER AVAILABILITY INFLUENCES SPECIES DISTRIBUTIONS ON SERPENTINE SOILS

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ABSTRACT

Serpentine soils are highly variable environments, but how this variability contributes to maintaining distinct distributions of species at small scales is unclear. We studied parapatric populations of *Layia jonesii* A. Gray, a rare serpentine endemic, and *L. platyglossa* (Fisch. & C. A. Mey) A. Gray, a wide-spread species, to understand how edaphic variability and competitive interactions maintain co-occurring, somewhat interfertile, relatives within serpentine environments. We analyzed soil characteristics along a serpentine hillside where *L. jonesii* and *L. platyglossa* are restricted to the top and bottom of the hillside, respectively. We used reciprocal transplants to investigate fitness differences on the hillside and a competition experiment to determine if species interactions also restrict the distribution of *L. jonesii*. The soil analyses showed the top of the hill is drier than the bottom, yet both locations are chemically indistinguishable. *Layia platyglossa* is better adapted to the edaphic conditions at the bottom of the hillside and *L. jonesii* is sensitive to competition in the bottom region. Differences in soil water availability, and potentially its effect on the competitive environment, likely contributes to maintaining the distribution of these species. Ionic stress is often cited as a driver of species' distributions within serpentine environments, but our research suggests soil water availability can be an important factor influencing the distribution of interfertile species on serpentine at small scales. Annual variation in precipitation may drive local shifts in species distributions through alteration of the competitive environment.

Key Words: Competition, edaphic adaptation, *Layia*, local adaptation, plant distributions, plant soil relations, serpentine endemic, water availability.

Divergent natural selection across heterogeneous environments causes ecological differentiation between closely related species or ecotypes of the same species (Kawecki and Ebert 2004). This adaptation to specific environmental conditions contributes to maintaining spatial distributions of taxa by preventing the successful colonization of migrants from one habitat to another (Nosil et al. 2005; Sobel et al. 2010). Because ecological differentiation can control the distribution of plant species, it also directly contributes to reproductive isolation by limiting gene flow between interfertile taxa (McNeilly and Antonovics 1968; Kawecki and Ebert 2004). Investigating ecological differentiation between closely related species or ecotypes of the same species helps us understand what environmental factors maintain the distribution of plants across landscapes as well as how this can lead to speciation (Clausen et al. 1941; Angert and Schemske 2005; Lowry et al. 2008).

Divergent adaptation to different soil environments can strongly influence the distribution of plant species and is an important factor driving plant evolution (Kruckeberg 1954, 1986; Cornwell and Grubb 2003; Rajakaruna 2004; Baldwin 2005; Yost et al. 2012). Soils are highly heterogeneous environments with significant variability in chemical and physical characteristics, which can drive divergent selection among

taxa (Ettema and Wardle 2002; Yost et al. 2012). In particular, soils that are considered harsh edaphic environments, either physically, chemically, or both, can be strong agents of natural selection (Rajakaruna 2004; Harrison and Rajakaruna 2011; Rajakaruna et al. 2014). Studies of plants growing on harsh soils derived from serpentine (Kruckeberg 1951; Main 1974; Brady et al. 2005; Wright et al. 2006), gypsum (Meyer 1986; Palacio et al. 2007; Moore et al. 2014; Escudero et al. 2015) and mine tailings (Antonovics 1968, 2006; McNeilly and Antonovics 1968) show local adaptation compared to neighboring soil types. Heterogeneity within a single soil type at small spatial scales can also provide sufficient variability for divergent selection (Lechowicz and Bell 1991; Yost et al. 2012; Chen and Schemske 2015).

Plants adapted to soils derived from ultramafic parent material (high in magnesium and iron, like serpentine) are often studied as a model system to understand how divergent environmental conditions can lead to differential adaptation, reproductive isolation, and speciation (Brady et al. 2005; Kay et al. 2011; Anacker 2014). Serpentine soils are harsh edaphic environments characterized by large magnesium to calcium ratios, elevated levels of toxic heavy metals, and deficient levels of plant essential macronutrients like phosphorus and potassium. Serpentine

also weathers quickly causing soils to be highly erodible and dry (Alexander et al. 2007). These harsh chemical and physical conditions are hypothesized to drive divergent selection, and therefore differential adaptation, of serpentine vs. non-serpentine taxa (Rajakaruna 2003, 2004; Brady et al. 2005; Kay et al. 2011; Wright and Stanton 2011).

Traditionally, studies of ecological differentiation on serpentine soils involve comparing species or ecotypes growing in discrete soil types (i.e., serpentine vs. non-serpentine soils), but serpentine environments are highly heterogeneous, ranging from shallow rock outcrops to deep grassland soils. This variability has the potential to drive divergent selection, and therefore divergent adaptation, even at small spatial scales *within* serpentine environments (Gram et al. 2004; Harrison et al. 2006; Alexander et al. 2007). Divergent adaptation within serpentine environments can lead to ecological differentiation among taxa, which can reinforce reproductive isolation and drive speciation, as well as foster species co-existence through niche partitioning (Silvertown 2004). For example, Yost et al. (2012) found that differential adaptation to edaphic variation across a serpentine hillside maintains distinct distributions and reproductive isolation between two closely related species in the genus *Lasthenia* (Asteraceae) Cass. Observing and investigating the distribution of close relatives within serpentine environments can help us understand which factors allow relatives to co-exist in close proximity, and yet studies investigating such co-occurrences are scarce. Serpentine soils also support high concentrations of rare, endemic species (Safford et al. 2005; Harrison and Rajakaruna 2011; Anacker 2014), therefore understanding how environmental heterogeneity influences distribution of plant species can provide insight into why these areas are biologically diverse.

Some serpentine endemic species face a trade-off between serpentine tolerance and competitive ability, and it has been hypothesized that the suboptimal conditions found in serpentine environments serve as a refuge from competition (Kruckeberg 1954; Grime 1977; Gram et al. 2004; Brady et al. 2005; Kazakou et al. 2008; Harrison and Rajakaruna 2011). Grasslands on serpentine soils are less productive, and therefore provide a less competitive environment, than most non-serpentine soils (Moore and Elmendorf 2011). Although there is evidence that the distribution of serpentine endemics may be controlled by competition at a broad scale, it is unclear how competitive interactions influence the distribution of serpentine endemics within serpentine environments at a fine scale.

The genus *Layia* (Asteraceae) Hook. & Arn. ex DC. consists of rare edaphic specialists, as well as more widespread edaphic generalists, and therefore provides a good system to investigate how ecological differentiation and adaptation to specific edaphic environments maintains species' distributions and contributes to speciation (Clausen 1951; Baldwin 2005, 2006). In California, rare edaphic specialists

like *L. jonesii* A. Gray and *L. discoidea* D.D. Keck (both restricted to serpentine soils) and *L. munzii* D.D. Keck (restricted to alkali soils) have extremely limited ranges. The genus also consists of widespread species, including *L. platyglossa* (Fisch. & C.A. Mey) A. Gray, which occurs through extensive portions of central and southern California in a wide variety of habitats and soils, and *L. glandulosa* (Hook.) Hook. & Arn., which occurs throughout the western United States and into Mexico in mostly sandy soils (Baldwin et al. 2012). Investigating edaphic factors that contribute to the wide spectrum of spatial distributions within this genus will help us assess the importance of these edaphic factors to species co-existence as well as speciation.

Here, we focused our investigation on *L. jonesii*, a rare serpentine endemic restricted to only a few populations in San Luis Obispo County, CA and *L. platyglossa*, a more common and widespread species. The widespread *L. platyglossa* is sister to a small clade containing *L. jonesii*, *L. munzii*, and *L. leucopappa*. In other words, while *L. platyglossa* is a close relative of *L. jonesii*, they are not sister species, but they do retain modest interfertility (Clausen 1951; Baldwin 2003). In San Luis Obispo, these two species often co-occur within a few meters of each other (parapatrically) on serpentine derived grassland hillsides (CCH 2017). At sites where the two species are known to co-occur, each species has a distinct, non-overlapping distribution. *Layia jonesii* occurs mainly on the top of the hillsides, where the mostly native vegetation is sparse and patchily distributed and where serpentine rock outcrops are exposed. *Layia platyglossa* occurs mainly on the bottom of the hillside where the vegetation is denser and composed of annual and perennial forbs as well as native and non-native grasses. Although the two species have distinct distributions at sites where they co-occur, there is little visible evidence of a sharp division of the two regions into environmentally distinct habitats. These species provide an opportunity to investigate how heterogeneity within a serpentine edaphic environment can contribute to the maintenance of distinct species distributions, and therefore can help broaden our understanding of how this heterogeneity can contribute to speciation and high levels of endemism in serpentine environments.

Soil chemical and physical properties, as well as the competitive environment, vary across small spatial scales within serpentine habitats and could influence the distribution of species (Alexander et al. 2007; Moore and Elmendorf 2011). Reciprocal transplants are a powerful way to test for fitness differences between regions within serpentine, and, when combined with a thorough analysis of the soil environment, transplants can help us understand which edaphic factors control the distribution of species due to differential adaptation (Nagy and Rice 1997; Angert and Schemske 2005; Sambatti and Rice 2006; Wright et al. 2006; Yost et al. 2012; Chen and Schemske 2015). Here, we use reciprocal transplants combined with a competition experiment (neighbor

removal) and a soil analysis to address the potential mechanisms that maintain the fine-scale spatial separation between *L. jonesii* and *L. platyglossa*. We tested four hypotheses to understand how fitness of the two species and soil characteristics differ along the hillside. We addressed the following hypotheses about the fitness of the two species across the hillside: (1) each species will have a higher fitness within its natural distribution than outside its natural distribution, and (2) the removal of neighbors will increase the fitness of *L. jonesii* outside its natural distribution at the bottom of the hill. We addressed the following hypotheses regarding the edaphic factors that could contribute to maintaining the unique distribution of *Layia*: (3) there are small-scale chemical differences between the soils in the two regions, and (4) there are small-scale physical differences between the soils in the two regions. It is important to note that these variables likely do not independently affect the distribution; rather, the distribution of *Layia* may be controlled by a combination of these factors.

METHODS

Study System

The genus *Layia* consists of 14 spring-flowering annual species native to California. Most species are self-incompatible, including *L. jonesii* and *L. platyglossa* (Clausen 1951). *Layia jonesii* and *L. platyglossa* are reliably distinguished morphologically by pappus shape. *Layia jonesii* has a pappus of short scales, whereas *L. platyglossa* has pappus of long bristles. *Layia jonesii* is endemic to San Luis Obispo County, CA on serpentine derived soils (Safford et al. 2005). The distribution of *L. jonesii* consists of only about 15 small, scattered locations from the city of San Luis Obispo north to Cayucos (CCH 2017). In contrast, *L. platyglossa* is a more widespread species ranging from Baja California to the northern coast ranges of California, and occurs on a range of soils, including serpentine (Clausen 1951; Baldwin et al. 2012). Herbarium records document the two species growing parapatrically (as adjacent populations) at multiple populations within San Luis Obispo County (CCH 2017).

At one of these locations, the two species occur within meters of each other on a serpentine-derived hillside in Reservoir Canyon Open Space (RC), San Luis Obispo County, CA (35.28490, -120.61830). This distribution is characteristic where *L. jonesii* and *L. platyglossa* co-occur. RC is part of a series of serpentine ridges that run parallel to the Santa Lucia Range, and are part of the Franciscan Complex, a coastal geologic formation consisting of mainly metamorphic substrates, including serpentine.

Reciprocal Transplant and Competition Experiment

To determine if either species is adapted to the specific edaphic environment on the region of the

hillside where it occurs, we performed a reciprocal transplant between *L. jonesii* and *L. platyglossa* at RC. We established four reciprocal transplant plots (0.5m x 0.5m) on a serpentine hillside where *L. jonesii* and *L. platyglossa* co-occur. Two plots were placed at the top of the hillside in the region where *L. jonesii* occurs, and two plots were placed at the bottom of the hillside in region where *L. platyglossa* occurs. The plots at the top of the hill were approximately 40m upslope from the plots at the bottom of the hill. In each of the four plots, we planted two single-seeded fruits (achenes or cypselsae, herein called seeds) from 16 maternal families of each species. Seeds were collected from RC during the previous season. In total, we planted 32 seeds (16 families) of *L. jonesii* and 32 seeds of *L. platyglossa* (16 families) for a total of 64 seeds per plot. We glued each seed to a color-coded toothpick to assist with tracking each plant through its life cycle. Each seed was randomly assigned a planting position within the plots. We planted the seeds in early November 2013.

To determine whether interspecific competitive interactions restrict the range of *L. jonesii* to the top of the hill, we conducted a competition experiment known as a neighbor removal experiment. In both of the transplant plots at the bottom of the hill, we established two smaller sub-plots (weeded plots) adjacent to the reciprocal transplant plots in which we planted two additional seeds from each of the same 16 maternal families of *L. jonesii*. On a weekly basis, we weeded out all plants except for the planted *L. jonesii* seedlings.

To document fitness across multiple life history transitions, we recorded germination, survival, and flowering time in the plots on a weekly basis until the end of the growing season. We quantified fitness using three measures: proportion of seeds germinated, proportion of germinated seeds that surviving to flowering, and viable seed set (total reproductive fitness). We quantified seed set by counting the number of viable seeds produced by each seed planted in both the reciprocal transplant and competition experiment plots. Seeds that failed to germinate were considered to have a fitness of zero. Dark-colored, swollen achenes were assumed to contain a viable seed. We also measured peak flowering time, which was considered to be when the highest proportion of open heads were present within the plots. An open head was defined as having at least one open disk floret.

We analyzed germination success and survival to flowering success in the plots using generalized linear models (GLM), and we analyzed viable seed set in the reciprocal transplant plots using a generalized regression with a zero-inflated Poisson distribution to account for many zero values within our dataset. We analyzed viable seed set in the competition plots using GLM. All analyses were performed with JMP (JMP 12.1.0, SAS Institute Inc., Cary, NC). To understand the effect of plot and maternal family on these analyses, we first ran our models by including

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Antimicrobial Synergies of the Digestive Juices Formed by Nepenthaceae Plants (2nd place High School division)



Justen Whittall, *Madroño* Editor, with Arjun (left) and Ayana (right)

- **Ayana Rose Wilmot, Oakwood School, Morgan Hill, CA:** Factors Affecting the Rate of Photosynthesis of California Lichens (1st place Middle School division)
- **Arjun Raiaram, Challenger School-Almaden, San Jose, CA:** The effect of different plants on firescaping (2nd place Middle School division)

2018 Santa Cruz County Science & Engineering Fair:

- **Emma Schaefer-Whittall, Scotts Valley High School, Scotts Valley, CA:**
Alkaloid Quantification of *Catharanthus roseus* and *Vinca major* and its Effects on Cell Viability (1st place High School division)
- **Isabel O'Malley-Krohn, Pacific Collegiate School, Santa Cruz, CA:**
Trees for the People:
Socioeconomic Distribution of Street Trees (2nd place High School division)
- **Caitlin Sullivan, Holy Cross School, Santa Cruz, CA:** Effects of X-Ray Radiation on Plants (1st place Middle School division)
- **Ashlyn McDaniel, Baymonte Christian School, Scotts Valley, CA:** Vinegar Stops Mold on Strawberries (2nd place Middle School division)



Justen Whittall with (starting from left) Ashlyn, Caitlin and Emma

Plot (nested within region) and Family (nested within species) as fixed effects (full model). When Plot or Family fixed effects were not significant ($P < 0.05$), we excluded them from our analysis and ran a reduced model. We report AICc values for full and reduced models, and our figures are reported using the model with the lowest AICc.

Soil Sampling

After the completion of the transplant experiment, we sampled soil from the four reciprocal transplant plots to characterize the soil chemistry at RC. We made three, 1-liter collections of soil from the top 15cm in each plot. The soil was tested at A&L Western Laboratories for organic matter percent, estimated nitrogen release (ENR, lbs/acre), phosphorus (Weak Bray and Olson Method, ppm), potassium (ppm), magnesium (ppm), calcium (ppm), sodium (ppm), soil pH, hydrogen (meq/100g), cation exchange capacity (CEC, meq/100g), and sulfur (ppm). We performed a principal component analysis with these soil characteristics using JMP.

To determine the physical characteristics of the soils at RC, we conducted one soil profile (pedon) description in the *L. jonesii* region (top) and one in the *L. platyglossa* region (bottom) of the hillside. Within each region, we classified the soil directly adjacent to a reciprocal transplant plot by digging a soil pit to the bedrock level and exposing a vertical face of the soil profile to examine the horizons (layers), as is best practice in soil science (Soil Science Division Staff 1993). We described the depth, rock fragment content (using a sieve), texture, and structure for each horizon in both soil profiles. Each pedon description is representative of its respective region (Soil Science Division Staff 1993).

We also calculated the available water holding capacity (AWHC) for each soil profile using standard calculations based on texture, soil depth, and the proportion of rock fragments (Soil Science Division Staff 1993; Schoeneberger et al. 2012). Soils store water in soil pores, and some of that water is available to plants to absorb and use. The available water holding capacity (AWHC) is the amount of water (in cm) that is potentially available to plants. The AWHC is calculated for each soil horizon using the following equation:

$$\left[\text{textural class AWHC} \left(\frac{\text{cm AWHC}}{\text{cm soil}} \right) \right] \\ \times [\text{depth of horizon (cm soil)}] \\ \times [1 - \text{prop.rock fragments}]$$

The values for each horizon are added to calculate the AWHC of the entire soil profile. Using USDA-NRCS convention, we did not include weathered bedrock horizons (Cr) in our AWHC calculations because these horizons consist of mainly weathered rocks that do not possess water storage potential

TABLE 1. Principal component analysis of chemical soil characteristics between the *Layia jonesii* region at the top and the *L. platyglossa* region at the bottom of the hill on a serpentine hillside in Reservoir Canyon. Bold values represent loading scores above 0.5. CEC is the cation exchange capacity and ENR is the estimated nitrogen release.

Soil character	PC1 (35.8%)	PC2 (31.5%)
Organic matter (%)	−0.1315	0.9322
ENR (lbs/A)	−0.1299	0.9166
Phosphorus – weak Bray (ppm)	0.8316	−0.2535
Phosphorus – Olsen method (ppm)	0.7637	−0.2012
K (ppm)	0.7925	−0.1463
Mg (ppm)	0.0826	0.9351
Ca (ppm)	0.8522	0.2401
Na (ppm)	−0.0032	0.6035
Soil pH	− 0.8690	−0.3383
H (meq/100g)	−8.29E-17	2.17E-16
CEC (meq/100g)	0.3116	0.9106
Sulfur (ppm)	0.6659	0.0555
Ca:Mg (%)	0.8242	−0.1388

(Soil Science Division Staff 1993). We also did not include the influence of rock fragments on soil horizons that contained <5% rock fragments by volume (Soil Science Division Staff 1993). All horizons in our two pedons had a clay texture. The AWHC for clay soils is 0.15 cm AWHC/cm soil (Kramer 1983).

RESULTS

Soil Characteristics

To characterize the chemical edaphic environment on the serpentine hillside, we conducted a principal component analysis of 13 chemical soil characteristics in both the *L. jonesii* (top of hillside) and the *L. platyglossa* (bottom of hillside) regions. The first two principal components describe 67.3% of the variation in soil characteristics between the two regions in RC (Table 1, Fig. 1). The analysis shows no chemical differentiation between the soils in the two regions. Soils from both regions have low levels of essential nutrients like phosphorus, potassium, and calcium along with high levels of magnesium (loading scores above 0.5), a pattern characteristic of serpentine soils (Alexander et al. 2007).

To characterize the physical edaphic environment on the serpentine hillside, we conducted a soil profile (pedon) characterization in both the *L. jonesii* and *L. platyglossa* regions. Both soil profiles show evidence of soil development by the presence of accumulated clay in the subsurface horizon (B_t). The presence of this horizon in profiles indicates that both soils are stable (Buol et al. 2003). The soils in both regions are of similar depth to weathered bedrock (top 20cm, bottom 25cm; Table 2), however, the soil at the top of the hill in the *L. jonesii* region contains a higher percentage of rock fragments in all solum horizons

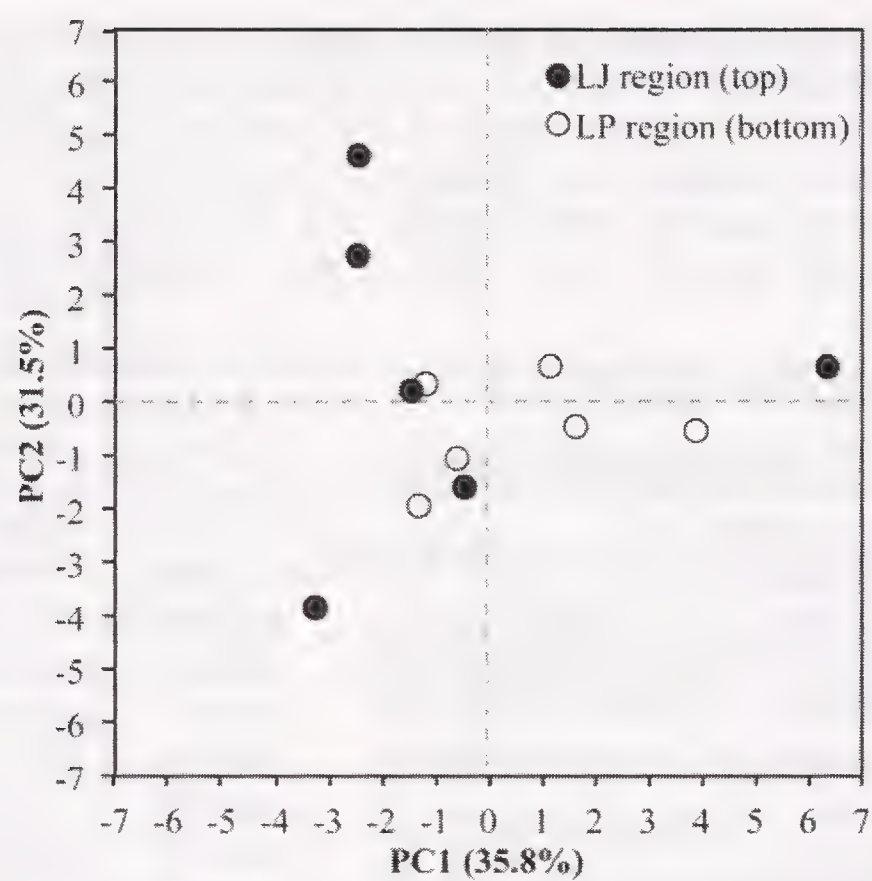


FIG. 1. Principal component analysis of the 13 chemical soil characteristics. The first two principal components (PCs) describe 67% of the variation in soil characteristics between the two regions at RC. The points in the plot above represent individual soil samples from the *L. jonesii* region at the top of the hill (black circles) and the *L. platyglossa* region at the bottom of the hill (white circles). See Table 1 for loading scores.

(horizons above the bedrock, C_r) compared to the soil in the *L. platyglossa* region.

The presence of a high percentage of rock fragments in the solum reduces the available water holding capacity (AWHC) of the soil. The water that is available to plants is held within the pores of the soil matrix, therefore, any factor that reduces the total volume of the soil matrix also reduces the available water holding capacity. Both rock fragments and soil depth can influence the total volume of the soil matrix (Hillel 2004). For example, shallow soils have less soil volume, therefore less available water holding capacity than deep soils. Similarly, rock fragments reduce the total volume of the soil matrix, therefore, rocky soils have less available water holding capacity than soils without rocks. The available water holding capacity of the soil in the *L. jonesii* (LJ) region is about half of the water holding capacity of the soil in the *L. platyglossa* (LP) region (soil in LJ region 1.29 cm of water; soil in LP region 3.75 cm of water; Table 2).

TABLE 2. Soil profile descriptions for soil pits at the *L. jonesii* (LJ) region at the top and the *L. platyglossa* (LP) region at the bottom of the hill. Available water holding capacity (AWHC) in cm of water for the entire soil profile is provided for each region.

Region	AWHC (cm)	Horizon	Depth (cm)	Rock fragments	Texture	Structure
LJ	1.29	A	0–1.5	<5%	Clay	Granular
		Bt	1.5–6	50%	Clay	Blocky
		Bt/C	6–20	65%	Clay	Blocky
		Cr	20+	-	-	-
LP	3.75	A	0–5	<5%	Clay	Granular
		Bt	5–25	<5%	Clay	Blocky
		Cr	25+	-	-	-

Reciprocal Transplant

We sought to determine if *L. jonesii* or *L. platyglossa* are differentially adapted to specific edaphic regions on the hillside at RC using a reciprocal transplant. To quantify plant fitness during the early portion of the life cycle, we tracked the germination success (LJ at top n = 62, at bottom n = 63; LP at top n = 63, at bottom n = 63) and survival to flowering success (LJ at top n = 21, at bottom n = 25; LP at top n = 20, at bottom n = 15) in the plots. The proportion germinating was higher in *L. jonesii* than *L. platyglossa* ($\chi^2 = 5.845$, P = 0.0156; Table 3; Fig. 2A). The proportion surviving to flowering of *L. jonesii* and *L. platyglossa* was not significantly different in the plots in both the top and bottom regions (Table 3; Fig. 2B). We also tracked the overall survival of germinated seedlings in the plots over time throughout the duration of the experiment. In the *L. jonesii* region (top of hillside), we observed that *L. platyglossa* had higher mortality than *L. jonesii* during the beginning of the experiment. During early April, the proportion of *L. platyglossa* seedlings surviving in the *L. jonesii* region dropped to 53% while the proportion of *L. jonesii* seedlings surviving in the same region remained at 100% (Fig. 3A, B). The overall survival of both species in the *L. platyglossa* region (bottom of hillside) was not different.

We used viable seed set of all individual seeds planted to determine the reproductive fitness of *L. jonesii* and *L. platyglossa* in the two regions on the hillside in RC (LJ at top n = 62, at bottom n = 63; LP at top n = 63, at bottom n = 63). Results from the reciprocal transplants showed the reproductive fitness of both *L. jonesii* and *L. platyglossa* was higher at the bottom of the hill in the *L. platyglossa* region than at the top of the hillside in the *L. jonesii* region (LJ at top 2.42 ± 3.84 mean viable seeds per individual, at bottom 15.28 ± 5.21 mean viable seeds per individual; LP at top 0.86 ± 3.04 mean viable seeds per individual, at bottom 5.26 ± 5.23 mean viable seeds per individual; $\chi^2 = 12.332$, P = 0.0004; Table 3; Fig. 2C). *Layia jonesii* showed a somewhat higher fitness in both regions on the hillside compared to *L. platyglossa* ($\chi^2 = 8.759$, P = 0.0031; Table 3; Fig. 2C).

TABLE 3. A summary of the generalized linear models (germination and survival to flowering analyses) and generalized regression (viable seed set) for the fixed effects of region (top or bottom of hill), species, region x species, plot (nested within region), and family (nested within species) on the germination, survival to flowering, and viable seed set of *L. jonesii* and *L. platyglossa* in the reciprocal transplant plots. The full model includes all fixed effects. The reduced model excludes plot or family when not significant ($\alpha = 0.05$) in the full model. The chi-squared value for the viable seed set analysis is the Wald chi-squared value. The AICc values are presented for all models. Values in bold indicate significant effects.

Fixed effect	df	Germination			Survival to flowering			Viable seed set						
		Full model		P	Full model		P	Full model		P				
		χ^2	AICc = 334.11		χ^2	AICc = 182.17		χ^2	AICc = 453.03					
											Reduced model		Reduced model	
											χ^2	P	χ^2	P
Region	1	0.048	0.8259	0.723	0.3951	1.489	0.2223	10.440	0.0012	12.332	0.0076			
Species	1	5.845	0.0156	1.599	0.2059	1.467	0.2254	16.593	<0.0001	8.759	0.0200			
Region x Species	1	1.508	0.2195	2.540	0.1110	1.193	0.2746	0.085	0.7704	0.062	0.5524			
Plot [Region]	2	54.800	0.0037	29.274	0.2988	-	-	2.693	0.2601	-	-			
Family [Species]	30	9.156	0.0103	2.177	0.3368	-	-	84.217	<0.0001	51.43	0.0088			

We also tracked flowering times of both species in the reciprocal transplant plots. In both regions, peak flowering time of *L. jonesii* occurred about a week earlier than *L. platyglossa*. Peak flowering time of both species occurred about a week earlier at the top of the hillside in the *L. jonesii* region than at the bottom of the hillside in the *L. platyglossa* region (Fig. 3A, B). Although *peak* flowering time was different between species and regions, a portion of each species' flowering cycles overlapped. For example, during peak flowering time of *L. jonesii* in the region at the top of the hill, individuals of *L. platyglossa* were blooming but the individuals in the plot had not yet reached peak flowering.

Competition Experiment

We sought to determine if the distribution of the rare serpentine endemic *L. jonesii* is constrained to the region at the top of the hillside because of interspecific competitive interactions at the bottom of the hillside. Results from the competition experiment at the bottom of the hillside showed that germination (LJ without weeding n = 63, with weeding n = 64) and survival to flowering (LJ without weeding n = 25, with weeding n = 26) of *L. jonesii* in plots at the bottom of the hillside were not significantly different for weeded and un-weeded plots ($\chi^2 = 0.003$, P = 0.95; and $\chi^2 = 0.05$, P = 0.81, respectively; Table 3; Fig. 2D, E). The *L. jonesii* individuals had a higher average reproductive fitness when neighbors were removed in the weeded portion of the plots than when neighbors were not removed (LJ without weeding 15.28 ± 17.25 mean viable seeds per individual; LJ with weeding 94.35 ± 53.9 mean viable seeds per individual; F = 4.49, P = 0.036; Fig. 2F). The increase in reproductive fitness of LJ in the weeded portion of the plot is likely due to decreased competition for resources such as water, nutrients, or light rather than increased pollinator efficiency due to increased visibility of floral heads to pollinators. Plants in the weeded portion of the plots were more robust and produced more floral heads. Removal of competitors was accompanied by an increased in the average number of floral heads per individual from 2.35 to 8.71.

DISCUSSION

Differential adaptation and edaphic specialization can control the distribution of plant species at a local scale (Lechowicz and Bell 1991; Yost et al. 2012). Heterogeneity *within* serpentine environments can potentially drive divergent selection and allow for the co-existence of close relatives (Kay et al. 2011; Yost et al. 2012). Studies investigating variability within serpentine environments can help us to further understand which edaphic factors influence distributions and co-existence of species at small scales. At RC, the distinct distribution of two species in the genus *Layia* on a serpentine hillside provided an opportunity to study how heterogeneity within

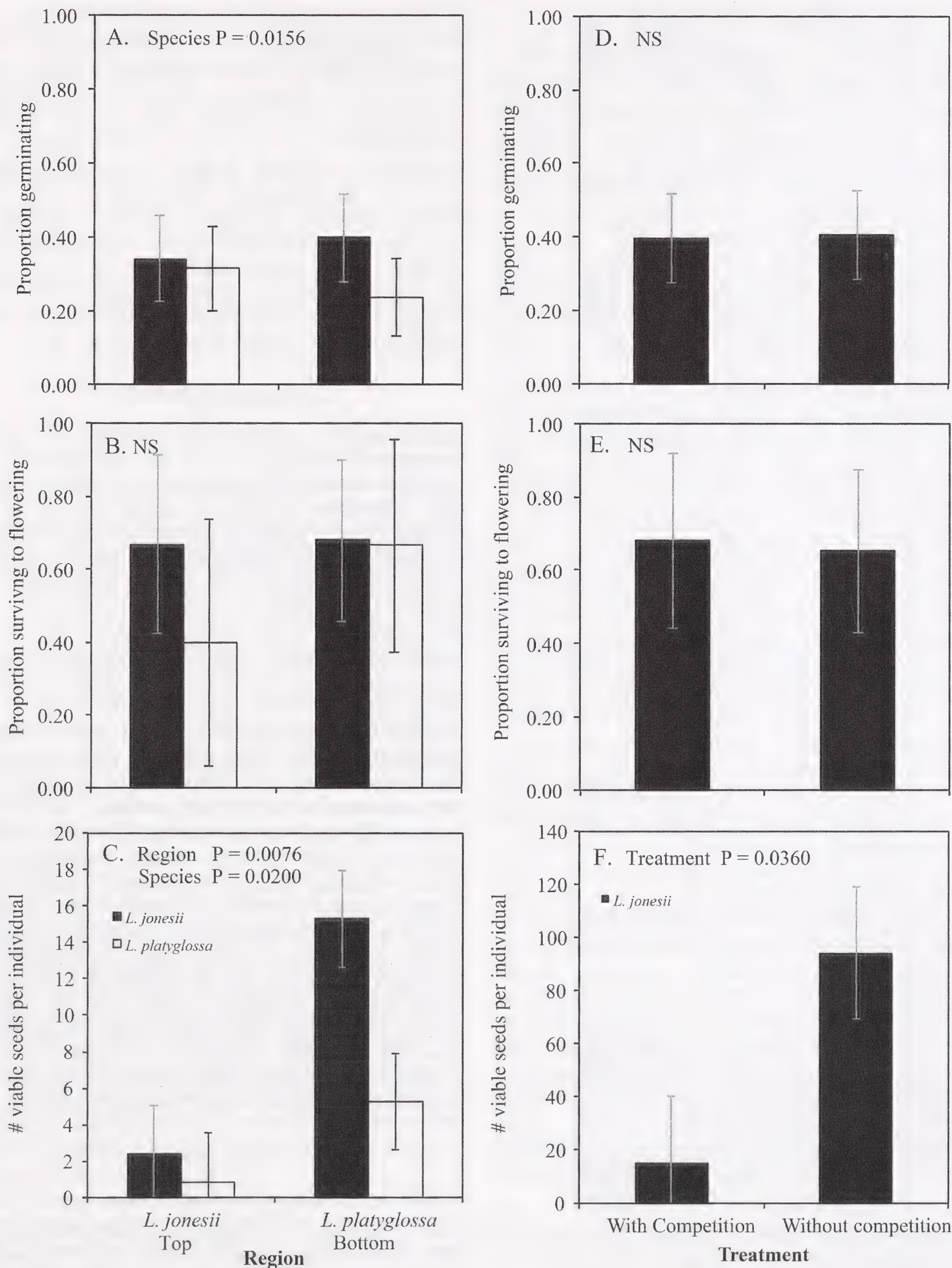


FIG. 2. Fitness parameters measured for *L. jonesii* (black bars) and *L. platyglossa* (white bars) during the reciprocal transplant and competition experiments. The left column shows the results from the reciprocal transplant experiment: (A) germination, (B) survival to flowering of germinated individuals, and (C) number of viable seeds produced per individual seed planted. The right column shows the results from the competition experiment: (D) germination, (E) survival to flowering of germinated individuals, and (F) number of viable seeds produced per individual seed planted. Bars in graphs (A), (B), (D), and (E) represent proportions. Bars in graphs (C) and (F) represent the average number of viable seeds produced by *L. jonesii* and *L. platyglossa* within each region or treatment. Only significant effects ($P < 0.05$) from the analyses are listed in each graph. Non-significance is denoted as NS. See Table 3 for a summary of the analyses. Error bars in (A), (B), (D), and (E) represent a 95% confidence interval. Error bars in (C) and (F) represent ± 1 SE.

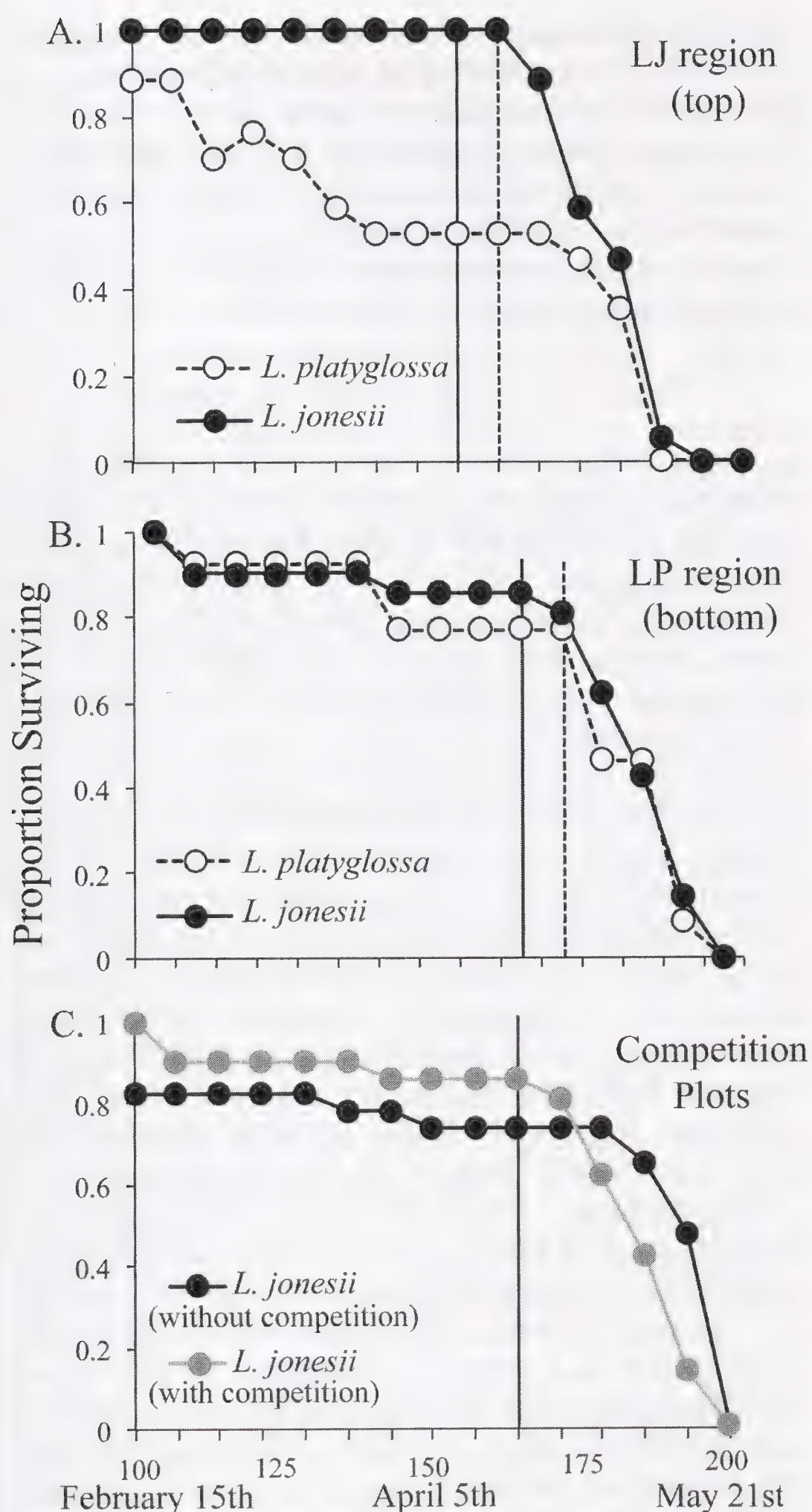


FIG. 3. Overall survival of germinated seedlings over time since planting of seeds in the transplant plots in the (A) *L. jonesii* region at the top of the hill, (B) *L. platyglossa* region at the bottom of the hill, and (C) competition plots (with and without weeding) in the *L. platyglossa* region at the bottom of the hill. The vertical solid line represents peak flowering time for *L. jonesii* and the vertical dashed line represents peak flowering time for *L. platyglossa* within the plots. In the competition plots, *L. jonesii* in plots with and without weeding had the same peak flowering time and it is denoted by a single solid vertical line.

serpentine environments can contribute to maintaining distinct distributions and to understand how these factors may contribute to high serpentine diversity. We found that soil water availability caused by differences in physical soil properties likely controls the distinct distribution of the two species.

Soil Environment

Because serpentine soils are chemically extreme, most studies of plant distributions within serpentine

environments focus on chemical properties of the soils and largely overlook important physical properties, such as soil depth, soil texture, and rock fragment content. These properties can have an important influence on water and plant relationships (Kramer 1983; Hillel 2004), and may be an important factor influencing plant distributions. Our study is one of the first to compare both chemical and physical properties of serpentine soils between two regions to understand how edaphic factors influence species' distributions.

Soils in the *L. jonesii* region near the top of the hill and the *L. platyglossa* region near the bottom of the hill were chemically indistinguishable, therefore, soil chemistry is not a factor contributing to the distinct distribution of *Layia* in RC. Although our study unexpectedly found the soils to be chemically similar, other studies have found the soil chemical environment is an important factor contributing to the coexistence of species within serpentine environments (Rajakaruna and Bohm 1999; Rajakaruna et al. 2003b). Yost et al. (2012) found that ionic differences along an edaphic gradient were an important factor driving differential fitness between closely related species of *Lasthenia*.

Although the soils are chemically similar, they are physically distinct. The soils near the top of the hill in the *L. jonesii* region contain a higher percentage of rock fragments in each horizon than soils at the bottom of the hill. Because of the high rock fragment content, soils at the top of the hill can hold about half as much water as the soils at the bottom of the hill. This means there is less soil water available to plants in the *L. jonesii* region. Because of its decreased water holding capacity, the *L. jonesii* region is expected to become drier earlier in the spring than soils in the *L. platyglossa* region. This phenomenon can easily be observed at RC and other similar sites, where the annual grasses and forbs near rocky outcrop regions flower and senesce earlier than plants in more densely vegetated grasslands regions. This accelerated phenology is likely a way for these individuals to escape impending seasonal drought (Heschel and Riginos 2005; Wu et al. 2010).

Spatial differences in soil water availability have been shown to be an important environmental mechanism influencing the distribution of species at both local and broad scales. Engelbrecht et al. (2007) showed drought sensitivity of various trees in tropical forest ecosystems controls the spatial distributions of species across a rainfall gradient in Panama. Differences in drought sensitivity can also contribute to maintaining distributions between closely related taxa at smaller spatial scales as well. Chen and Schemske (2015) found that variation in soil moisture is an important factor influencing the distribution of two closely related species in the tropical ginger genus *Costus*. Within serpentine environments, soil water availability has also been linked to divergent adaptation. Temporal differences in water availability between a seep and riparian

TABLE 4. A summary of the general linearized models (GLM) for the treatment (non-weeded vs. weeded plots), plot (nested within treatment), and family on the germination, survival to flowering, and viable seed set of *L. jonesii* in the competition plots. The full model includes all fixed effects. The reduced model excludes plot and family when not significant ($\alpha = 0.05$) in the full model. The AICc values are presented for all models. Values in bold indicate significant effects.

Fixed effects	df	Germination			Survival to flowering			Viable seed set			
		Full model AICc = 192.84		Reduced model AICc = 175.19		Full model AICc = 98.62		Full model AICc = 864.58		Reduced model AICc = 846.97	
		χ^2	P	χ^2	P	χ^2	P	F	P	F	P
Treatment	1	0.003	0.9570	0.011	0.9137	0.056	0.8124	4.188	0.0431	4.493	0.0360
Plot [Treatment]	2	1.386	0.5000	-	-	24.476	0.0401	0.556	0.5747	-	-
Family	15	22.324	0.0996	-	-	3.425	0.1804	1.541	0.1033	-	-

environment help to maintain divergent populations of a serpentine endemic sunflower (Sambatti and Rice 2006). Differential responses of two species of *Lasthenia* to water stress suggest water availability could be an important factor influencing the distinct distributions, and therefore reproductive isolation, of closely related species within serpentine environments (Rajakaruna et al. 2003a; Yost et al. 2012).

Selective Pressures within Serpentine

Reproductive fitness data and survival data suggest *L. platyglossa* is better adapted to the region near the bottom of the hillside. Reproductive fitness data (viable seed set) from the reciprocal transplant showed *L. platyglossa* was less fit in the rocky *L. jonesii* region near the top of the hillside than in its own region near the bottom of the hillside (Fig. 2C). Overall survival data also showed that *L. platyglossa* was less fit near the top of the hillside than at the bottom. The dry conditions found near the top of the hillside likely explain the fitness differences we detected between the two regions. Because the top of the hillside becomes drier earlier in the season, *L. platyglossa* in the transplant plots may not have been able to allocate sufficient resources to survival and reproduction under drought stress conditions, and this may limit the potential for the species to expand into the *L. jonesii* region. Future work in the greenhouse could directly test this hypothesis.

Unexpectedly, the reciprocal transplant did not support the idea that *L. jonesii* is best adapted to the edaphic conditions present near the top of the hillside in its own region. Although we observed the distribution of *L. jonesii* constrained to the top of the hill during 2013, the year before our transplant, our results indicate *L. jonesii* had a higher fitness near the bottom of the hill in the *L. platyglossa* region. These results could be related to the extreme drought that coincided with our reciprocal transplant in spring 2014, which was California’s worst drought on record (Swain et al. 2014). During spring 2014, we observed that the natural distribution of *L. jonesii* expanded out from the rock outcrop region at the top and into the grassland region toward the bottom of the hillside. Extreme drought likely reduced the survival of many annual species in the grassland region, especially non-native grasses, which have been found to be especially sensitive to changes in precipitation on serpentine soils (Hobbs et al. 2007). Therefore, competition may have been lower in the grassland region during the drought than in previous years. Results from our competition experiment showed that *L. jonesii* is sensitive to competition from surrounding species. Reduced competition caused by drought may have allowed the *L. jonesii* individuals in our reciprocal transplant plots to take advantage of the more conducive growing environment, with higher water availability, found in soils in the grassland region near the bottom of the hill. The hypothesis that increased rainfall can lead to

increased productivity, and therefore increased competition on serpentine soils, is supported by other studies. Harrison et al. (2006) found that increased rainfall and the corresponding increased productivity was negatively correlated with serpentine endemic richness on serpentine rock outcrops in California. This result supports our hypothesis that increased fitness of *L. jonesii* in the grassland region could be due to reduced rainfall, and therefore reduced competition.

Reduced competitive ability of some serpentine endemics may be due to an evolutionary trade-off between serpentine tolerance and competitive ability (Kruckeberg 1954; Grime 1977; Kazakou et al. 2008; Kay et al. 2011). It has been hypothesized that serpentine soils serve as a refuge from competition for many serpentine endemics that may be poor competitors off serpentine (Kruckeberg 1954; Gram et al. 2004; Brady et al. 2005; Alexander et al. 2007). Our results suggest that competitive interactions could influence the spatial distribution of serpentine endemics at an even finer scale. The dry rock outcrop habitats patchily distributed on serpentine soils may serve as refugia from competition for endemic species within the serpentine environments (Gram et al. 2004). If non-endemic competitors are able to tolerate the chemical extremes of serpentine, they might not also be able to tolerate drought. Therefore, heterogeneity of water availability could contribute to maintaining biodiversity (Silvertown 2004).

Our anecdotal observation of *L. jonesii* expanding its local range during a drought year suggests there are very complex interactions between biotic and abiotic factors in this system. The distribution of *L. jonesii* and *L. platyglossa* may be dynamic from year to year, based on annual environmental conditions and interactions between species. This dynamic distribution pattern suggests natural selection varies from year to year based on climatic fluctuations. These inter-annual fluctuations may maintain the distribution of the two populations on serpentine-derived hillsides, where the expansion of the rare, and potentially more drought tolerant serpentine endemic *L. jonesii*, is favored during dry years, but expansion of the common *L. platyglossa* is favored during wet years. Our results are consistent with previous studies that suggest differences in drought tolerance can influence the co-existence and the distribution, and therefore reproductive isolation, of taxa or ecotypes within heterogeneous serpentine environments (Gardner and Macnair 2000; Hughes et al. 2001; Rajakaruna et al. 2003a; Sambatti and Rice 2006).

There are other examples of widespread members of the genus *Layia* co-occurring with rare congeners. For example, *L. platyglossa* co-occurs with the rare *L. munzii* D.D Keck on alkali soils in eastern San Luis Obispo County, California. These types of co-occurrences may be maintained by mechanisms similar to those reported here, such as differential adaptation to specific edaphic environments at a local scale. Investigating these kinds of co-occurrences

can provide insight into how rare species are maintained near interfertile and widespread relatives.

Flowering Time Differences

We also found evidence that peak flowering time for *L. jonesii* occurs earlier than *L. platyglossa*. In all plots, peak flowering time for *L. jonesii* occurred about a week earlier than *L. platyglossa*. Earlier flowering likely allows *L. jonesii* to complete its lifecycle and reproduce before the onset of the most intense period of seasonal drought negatively impacts the reproductive fitness of this species. We also observed that both species flowered earlier in the drier rock outcrop habitat than the grassland habitat. In this case, earlier flowering time of both species may be a plastic response to the dry conditions of the rock outcrop habitat. These patterns suggest that both genetic and environmental factors may control the flowering time of *L. jonesii* and *L. platyglossa*.

Drought can drive selection for earlier flowering times that shorten the life cycle and allow for reproduction before the onset of drought (Sherrard and Maherali 2006; Wu et al. 2010; Franks 2011). Species that are unable to complete their life cycle before the onset of seasonal drought or that cannot effectively allocate resources to reproduction under drought stress will likely have a lowered fitness on dry soils (Chaves et al. 2003; Wu et al. 2010). Because drought can be a driver of selection for earlier flowering times, especially on dry serpentine rock outcrops, the rare serpentine endemic *L. jonesii* may be under strong selection for earlier flowering time. This is consistent with other studies that found some serpentine ecotypes flower earlier than non-serpentine ecotypes (O'Dell and Rajakaruna 2011). Although earlier flowering is associated with some serpentine ecotypes and taxa, flowering time shifts are not always associated with serpentine tolerance (Schneider 2017).

CONCLUSION

Edaphic factors influence the distribution of plant species, even at small scales. We found evidence that the distinct distribution of *L. jonesii* and *L. platyglossa* on a serpentine-derived hillside during our study period is maintained by physical, not chemical, edaphic factors. *Layia platyglossa* likely does not expand into the rock outcrop habitat because it is too dry, and the distribution of *L. jonesii* generally may be constrained by competitive interactions from surrounding species. Ionic stress is often cited as a driver of species' distributions within serpentine environments (Rajakaruna and Bohm 1999; Rajakaruna et al. 2003b), but our research suggests soil water availability is also an important factor influencing the distribution of species. We also observed a local population expansion of *L. jonesii* during an extreme drought, suggesting that temporal selection due to soil water availability may control

the boundaries of the observed distribution. Spatial variation in soil moisture is likely an important factor influencing the distribution of species on serpentine hillsides, and annual variation in precipitation may drive local range shifts in species distributions from year to year through alteration of the competitive environment. Investigating dynamic distributions of interfertile plant species helps us understand how adaptation to different soil water availability regimes can directly influence species co-existence, reproductive isolation, and speciation.

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STRUCTURE AND COMPOSITION OF SUBALPINE CONIFERS IN THE EMERALD LAKE WATERSHED, SEQUOIA NATIONAL PARK, CALIFORNIA

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ABSTRACT

The Emerald Lake watershed forms a subalpine basin in the upper drainage of the Marble Fork of the Kaweah River in Sequoia National Park, California, with elevations ranging from 2804–3416 m. Five conifer species are present in the basin, with western white pine (*Pinus monticola* Douglas ex D. Don) as the most dominant species forming 71% of the stems and 82% of basal area censused in 1985 and 1986. Lodgepole pine (*Pinus contorta* Loudon subsp. *murrayana* (Grev. & Balf.) Critchf.) was the second most dominant in the watershed as measured by numbers of individuals and basal area, but was almost entirely restricted to mesic bench and wet meadow habitats in the lower elevations of the watershed. Foxtail pine (*Pinus balfouriana* Grev. & Balf.) comprised 9.5% of the stems and 13.7% of the basal area within the basin, but was largely present on higher north-facing ridgelines where it formed 52% of stems and 65% of basal area. Jeffrey pine (*Pinus jeffreyi* Grev. & Balf.), and red fir (*Abies magnifica* A. Murray bis var. *critchfieldii* Lanner) were present in small numbers in the lower basin in wet meadow and/or mesic granite bench habitats.

Key Words: Emerald Lake, *Pinus balfouriana*, *Pinus contorta* subsp. *murrayana*, *Pinus jeffreyi*, *Pinus monticola*, Sequoia National Park, subalpine conifer, treeline.

Subalpine and alpine basins of the Sierra Nevada provide significant ecosystems not only for their biodiversity, but also for monitoring environmental impacts of global change including higher temperatures, altered patterns of snowmelt, and the deposition of anthropogenic nitrogen and phosphorus (Millar and Rundel 2016). These high mountain ecosystems are sensitive to small changes in growing season conditions of temperature and water availability, and their stability impacts hydrological conditions which provide a critical resource for downstream agriculture and urban development in California (Bales et al. 2006; Maurer 2007; Trujillo et al. 2012). Of particular concern, therefore, is the potential for alterations of the hydrology and thermal conditions in the Sierra Nevada that may impact the length of growing seasons and water availability and through such changes the community structure and productivity of high mountain ecosystems. Such changes may already be occurring. Data from vertebrate studies have indicated significant upward elevational range shifts for a number of species of birds and mammals over the past century (Moritz et al. 2008; Tingley et al. 2009), and subalpine plant species are expected to respond in a similar manner (Millar and Rundel 2016). Subalpine conifers, which form the major part of the basin biomass, represent a critical element for the stability of community and food web structure (Millar and Rundel 2016).

Studies of forest demography in the Sierra Nevada, as well as across the western United States, have reported an increase in overall mortality rate

over recent decades in species of both *Pinus* and *Abies* (van Mantgem and Stephenson 2007; van Mantgem et al. 2009). However, the magnitude of changes in mortality rate declined with increasing elevation, and there was no significant increase in mortality in subalpine plots (van Mantgem and Stephenson 2007). Concern about patterns of increasing mortality led Stephenson and van Mantgem (2005) to point out the need for increased baseline sampling efforts to definitively detect changes at high elevations.

The constrained size of the Emerald Lake watershed provided an opportunity for a detailed demographic survey of the size distribution and habitat of subalpine conifers. The objective of these data, when first collected decades ago, was to provide a complete census and measurement of every individual tree growing within the watershed and to categorize tree population and community structure within the physiographic habitats of the watershed. In addition, samples were collected to quantify branch tip growth and needle retention, covering a 5-yr period predating the study as a measure of interannual changes in growth conditions for the three important pine species. These data thus provide a rich baseline source of information for future studies.

METHODS

Study Site

The Emerald Lake watershed in Sequoia National Park has served for more than three decades as a



FIG. 1. Emerald Lake Basin, Sequoia National Park, with Emerald Lake (2804 m) in the foreground and looking up to Alta Peak (3415 m). National Park Service photo by Rick Cain.

primary study site to understand the hydrology, ecology, and biogeochemistry of a representative subalpine basin in the southern Sierra Nevada (Fig. 1). The watershed, 120 ha in area, covers an elevational range from 2804 m at Emerald Lake to 3416 m at the summit of Alta Peak (Fig. 2). The Emerald Lake Basin is a glacial cirque, carved from granitic parent material and representative of many subalpine and alpine basins in the Sierra Nevada with lakes exhibiting weakly buffered calcium bicarbonate surface waters (Williams and Melack 1991). The climate of the Emerald Lake watershed is typical of the Mediterranean-type regime of the southern Sierra Nevada, with 75–90% the annual precipitation falling as snow in the winter months (Stephenson 1988). Based on limited long-term data on precipitation levels, mean annual precipitation is about 1600 mm, but amounts are highly variable between years (Sickman et al. 2003a). Snowmelt typically begins in April with peak flow of runoff water occurring in June (Sickman et al. 2003b). Summers are generally dry except for an occasional convective storm associated with monsoonal air masses from the east (Sadro and Melack 2012).

Bedrock exposed by glacial scouring and frost action covers nearly half of the surface area of the Emerald Lake watershed, with the remaining half covered by talus and thin soils in approximately

equal proportions, with soils present over only about 20% of the watershed surface (Sisson and Moore 1984; Tonnessen 1991), vascular plant cover is relatively sparse. Thin soils and fractures in the granite surface, however, do support a wide distribution of both wet and dry meadow communities throughout the basin. Furthermore, extensive woody shrub growth in local areas of the lower basin appear where soils are more developed and in rocky fracture lines across the basin where soil and moisture are available. Because of the rocky nature of the watershed, plant cover is highly restricted, although there are open stands of subalpine conifers as well as willow thickets (Rundel 2015), wet and dry meadows, and scattered plant growth in rock crevices, fellfields, and areas of colluvium. The flora is surprisingly large considering the limited area of developed soil profiles, with 202 vascular plant species present (Rundel et al. 2009).

Intensive research on hydrology and biogeochemical cycling in the Emerald Lake watershed began in 1984, with funding from the California Air Resources Board, to address concerns related to the influence of atmospheric inputs of nutrients to basin processes and ecosystem structure (Tonnessen 1991). Watershed ecosystem studies at this site have continued up to the present day and have provided detailed (and in many cases) long-term and continuing databases to

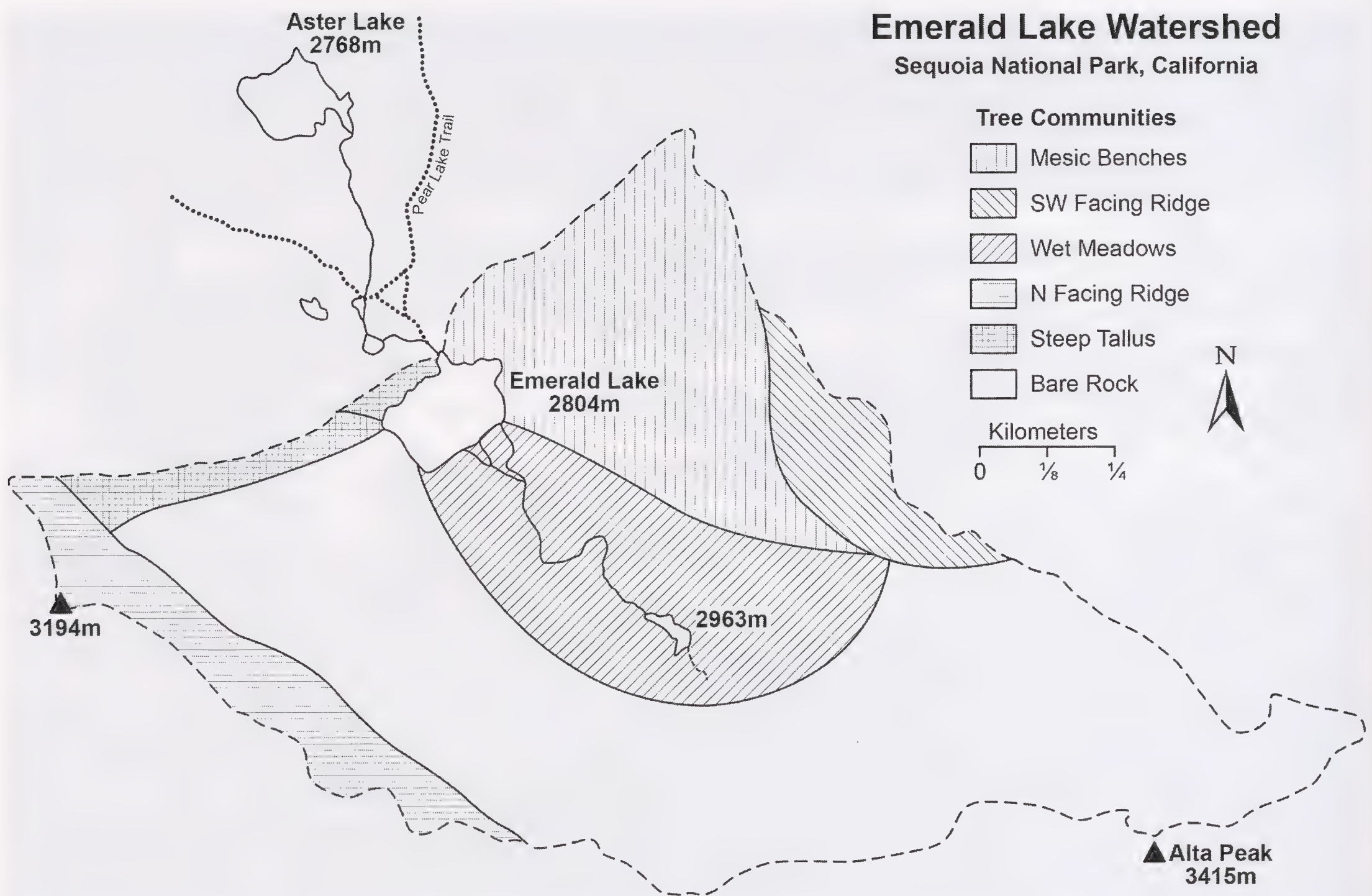


FIG. 2. Map of the Emerald Lake watershed showing the major physiographic divisions.

understand hydrologic flow and water balance (Kattelman and Elder 1991; Huth et al. 2004), nutrient enrichment (Sickman et al. 2003a, b), solute chemistry of snowmelt and runoff (Williams and Melack 1991), nitrogen fluxes and transformations (Miller et al. 2009), and models of hydrochemical response (Wolford et al. 1996; Wolford and Bales 1996; Meixner et al. 2004).

Field Measurements

A complete field census of all trees in the Emerald Lake watershed was carried out over the summers of 1985 and 1986. Each tree was identified, measured for diameter at breast height (dbh) to the nearest 1 cm. For saplings that did not reach breast height, the diameter measured was that of the stem base. No seedlings or small saplings of any species were found with basal diameters <5 cm. Stems were roughly mapped within each of five physiographic community habitats identified within the watershed. Habitats were determined by a combination of criteria, including physiographic position, substrate, moisture availability, exposure, and community composition, that together defined distinct assemblages. These habitats were mesic bench, wet meadow, southwest-facing ridgeline, north-facing ridgeline, and steep talus slopes. A major portion of the watershed area was bare rock without individual tree stems. The relationship between dbh and height was measured in 65 stems of *Pinus monticola* Douglas ex D. Don

growing in permanent plots established in the wet meadow and mesic bench habitats.

Phenological patterns of vegetative and reproductive growth were followed over four growing seasons to provide a measure of interannual variation in growth characteristics for three pine species. In October and November 1986, 20 actively growing branches collected from five individuals each of *Pinus balfouriana* Grev. & Balf., *P. contorta* subsp. *murrayana*, and *P. monticola* were harvested to provide quantitative data on the relative growth of branch tip elongation and biomass over the past five growing seasons. Measures of needle length, twig length, and biomass per growing branch tip were made for the years 1982–1986. Numbers of living needle fascicles and fascicle scars were counted for the past five growing seasons to evaluate needle retention over five growing seasons.

RESULTS

Tree Distribution and Composition

The conifer community of the Emerald Lake Basin included 1206 stems with five species present in the 120 ha area of the watershed. These were *Pinus monticola* (western white pine), *P. contorta* subsp. *murrayana* (lodgepole pine), *P. balfouriana* (foxtail pine), *P. jeffreyi* Grev. & Balf (Jeffrey pine), and *A. Murray bis magnifica* var. *crichfieldii* Lanner (red fir).

TABLE 1. Total tree density and basal area within each of the major physiographic habitats of the Emerald Lake watershed. Relative values in percent for the entire watershed are shown in parentheses.

	Area (ha ²)	Stems	Stem density (no. ha ⁻¹)	Basal area (m ²)	Basal area density (m ² ha ⁻¹)
Bare rock	59.6 (49.7%)	0	0.0	0	0.0
Mesic bench	23.5 (19.6%)	581 (48.2%)	24.7	89.0 (51.1%)	4.2
SW-facing ridge	8.9 (7.4%)	300 (24.9%)	33.8	44.3 (25.4%)	7.9
Wet meadow	19.1 (15.9%)	100 (8.3%)	5.2	5.9 (3.4%)	0.3
Steep talus	3.2 (2.7%)	57 (4.7%)	17.6	1.7 (1.0%)	0.5
N-facing ridge	5.6 (4.7%)	168 (13.9%)	29.8	33.3 (19.1%)	3.7
Total watershed	120 (100)	1206 (100)	10.1	174.2 (100)	1.45

The conifer community has a mean density of 10.1 trees ha⁻¹ for the entire watershed. On a basal area basis, the watershed contained 174.5 m² of tree basal area, or 1.45 m² ha⁻¹. These figures on density and basal area are misleading as more than half of the watershed is treeless and covered by bare granite outcrops (Fig. 1).

Breaking down the watershed's conifer community by slope exposure, 73.1% of trees were located on southwest-facing exposures. Northwest and north facing exposures contained 8.3% and 18.7%, respectively, of the basin trees. These values were close to the relative basal area present on each of these three exposures (Table 1). West facing exposures below Alta peak were smooth granite outcrops presenting little to no available habitat for tree establishment, while the orientation of the basin provide few areas with east-facing exposure.

The most abundant tree species in the watershed was western white pine with 71.2% (859 trees) of the total conifer community (Table 2). Next in abundance were lodgepole pine with 16.7% (202 trees) and foxtail pine with 9.5% (114 trees) in the basin. Only small numbers of red fir (20 trees) and Jeffrey pine (11 trees) were present. The distribution of relative basal area among the five conifers generally followed the relative density, but with increased relative dominance by western white pine (82.4%) and foxtail pine (13.7%).

The distribution of stems of western white pine by size-class displayed a typical inverse J-shape distribution with many smaller trees and relatively few large trees (Fig. 3A). Nearly 60% of the stems had a dbh of 15 cm or less. However, there seemed to be a secondary small peak of tree abundance for stems above 50 cm dbh, suggesting lower rates of mortality for these larger and hypothetically older trees. Some caution must be used in this interpretation since size is not a strong correlate of age in subalpine conifers (LaMarche and Mooney 1972; Brunstein and Yamaguchi 1992). Despite the high elevation and rocky substrate of the watershed, western white pine stems reached diameters up to 195 cm, with 70 trees with dbh >100 cm. All but nine of these occurred on the mesic bench habitat, with six on the north-facing ridge and three in the wet meadow area.

Lodgepole pine also exhibited an inverse J-shape distribution of size-class with many small trees and only very few stems reaching dbh of 50 cm or more (Fig. 3B). More than 60% of the stems were in the 15 cm or smaller dbh class. There were four trees over 50 cm over in diameter, with the largest individual in the watershed having a dbh of 97 cm.

The size-class distribution of foxtail pine in the watershed was heavily weighted toward stems of larger size compared with western white pine (Fig. 3C). There were proportionally fewer stems in the

TABLE 2. Distribution of trees and tree basal area within the entire Emerald Lake watershed and within each of the five physiographic habitats. Relative values in percent for each habitat are shown in parentheses.

	<i>P. monticola</i>	<i>P. contorta</i>	<i>P. balfouriana</i>	<i>P. magnifica</i>	<i>P. jeffreyi</i>	Total
Frequency (stems)						
Total watershed	859 (71.2%)	202 (16.7%)	114 (9.5)	20 (1.7%)	11 (0.9%)	1206
Mesic bench	419 (72.1%)	138 (23.8%)	0	13 (2.2%)	11 (1.9%)	581
SW-facing ridge	238 (79.3%)	36 (12.0%)	19 (6.3)	7 (2.3%)	0	300
N-facing ridge	77 (45.8%)	3 (1.8%)	88 (52.4)	0	0	168
Wet meadow	72 (72.0%)	23 (23.0%)	5 (5.0)	0	0	100
Steep talus	53 (93.0%)	2 (3.5%)	2 (3.5)	0	0	57
Basal area (m ²)						
Total watershed	143.8 (82.4%)	6.1 (3.5%)	23.9 (13.7%)	0.7 (0.4%)	0.7 (0.4%)	174.5
Mesic bench	83.4 (93.7%)	5.1 (5.7%)	0	0.4 (0.6%)	0.1 (<0.1%)	89.0
SW-facing ridge	43.3 (94.0%)	0.6 (1.4%)	2.1 (4.5%)	0.1 (0.1%)	0	46.1
N-facing ridge	11.5 (34.6%)	0.1 (0.2%)	21.7 (65.1%)	0	0	33.3
Wet meadow	5.2 (91.4%)	0.4 (6.5%)	0.2 (2.1%)	0	0	5.9
Steep talus	1.6 (93%)	0.06 (3.5%)	0.06 (3.5%)	0	0	0.2

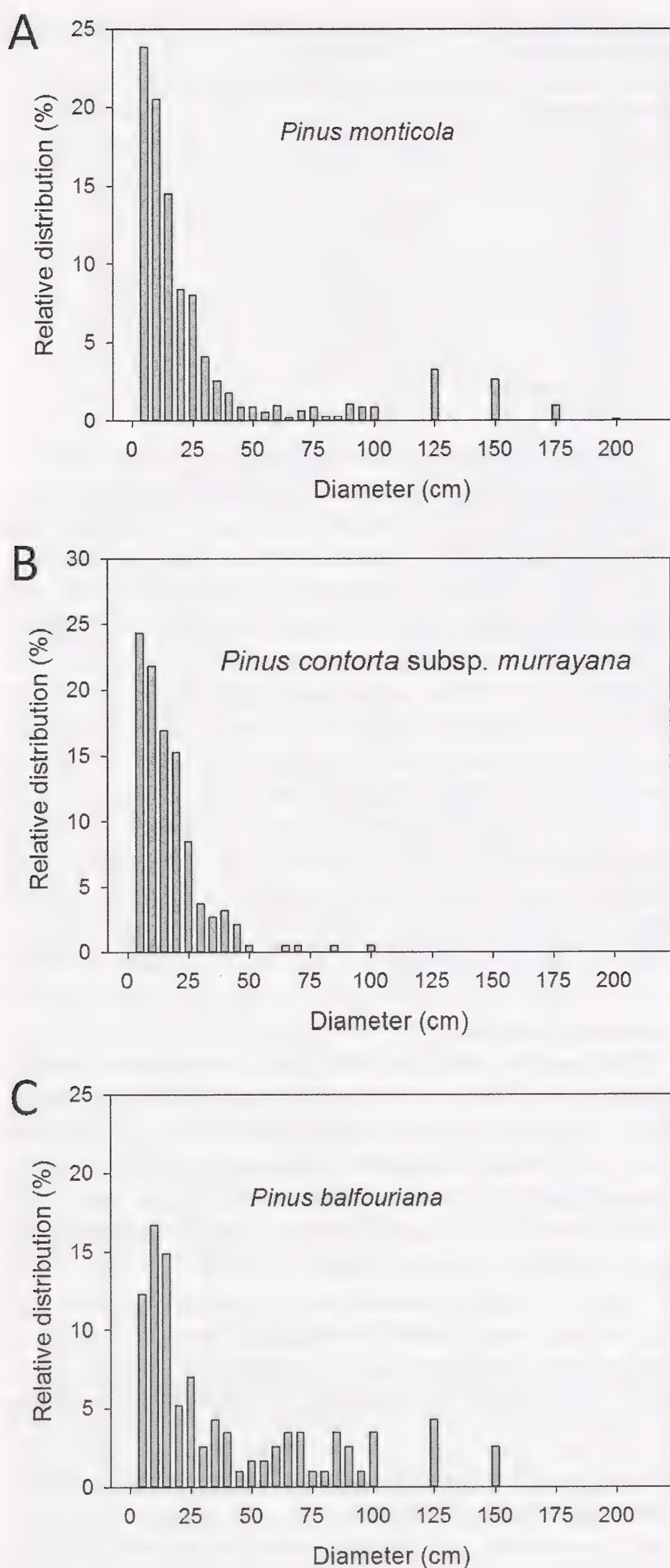


FIG. 3. Relative distribution by diameter class for *Pinus balfouriana*, *P. contorta* subsp. *murrayana*, and *P. monticola* in the Emerald Lake watershed. Large trees were measured to their dbh in cm and plotted here into their 5 cm class of diameters.

smaller dbh classes of <15 cm, although reproduction was taking place as these comprised over 40% of all stems. There were six trees that reached a dbh >100 cm, with the largest tree having a dbh = 146 cm.

Jeffrey pine was only represented by 11 stems, all of these growing at lower elevations in the mesic bench habitat. All of these had a dbh of 15 cm or less.

Red fir was also rare in the watershed, represented by just 20 stems with two-thirds of these growing in the mesic bench habitat. Three-quarters of the stems were in the small dbh classes of 15 cm or less, but there were three stems that had diameters of 31–47 cm, all in the mesic bench habitat, which likely represent an earlier period of establishment.

To assess patterns of tree distribution within the watershed beyond simple slope exposure, the Emerald Lake watershed was categorized into six physiographic habitats (Fig. 2). Approximately 60% of the watershed area, including all of the steep slopes below Alta Peak, was bare granite outcrops that lacked trees. The largest habitat type supporting trees was formed by mesic rock benches extending from Emerald Lake itself southeast along several input streams to about 3000 m elevation. These covered 19.6 ha (23.5%) of the basin. Wet meadow habitats spread across the north central area of the watershed at lower elevations covered 19.1 ha, (15.9%) of the area. The southwest-facing ridgeline along portions of the eastern boundary of the watershed covered 8.9 ha (7.4%) of the basin. The north-facing ridgeline along much of the upper southwestern boundary of the watershed covered 5.6 ha (4.7%) of the basin, while steep talus slopes along the northwest margin of the basin covered 3.2 ha (2.7%) of the watershed.

Breaking tree distribution down by habitat type, 48.2% (581 trees) were located in the mesic bench area of the watershed (Table 1). These accounted for 51.1% (89 m²) of the total basal area of trees in the watershed. For this habitat, mean tree density was 24.7 trees ha⁻¹ with a mean basal area of 4.2 m² ha⁻¹. Western white pine was the dominant species but nearly one quarter of trees present were lodgepole pine. This habitat at lower elevations and milder conditions within the watershed was home to two-thirds of the red fir and all of the Jeffrey pine. By contrast, there were no foxtail pines in this habitat.

Next in importance for tree distribution among the habitats was the southwest-facing ridge community, which despite its relatively small area included 24.9% (300 trees) of watershed stems and 25.4% (44.3 m²) of watershed basal area. Mean tree density was 33.8 trees ha⁻¹ with a mean basal area of 7.9 m² ha⁻¹. This habitat was dominated by western white pine, which made up 79.3% of stems and 93.8% of basal area.

The north facing ridgeline was home to 13.9% (168 trees) of watershed stems, with 19.1% (33.3 m²) of watershed basal area. Mean tree density was 29.8 trees ha⁻¹ with a mean basal area of 3.7 m² ha⁻¹. This was the favored habitat of foxtail pine in the basin with almost 89% of all of the foxtail pines occurring on this north-facing ridgeline. This species accounts for 52.4% of stems and 65% of the basal area for this habitat, with western white pine making up almost all of the remaining basal area.

The wet meadow habitat included 8.3% (100 trees) of watershed stems, with 3.4% (5.7 m²) of watershed basal area. Mean tree density was 5.2 trees ha⁻¹, with

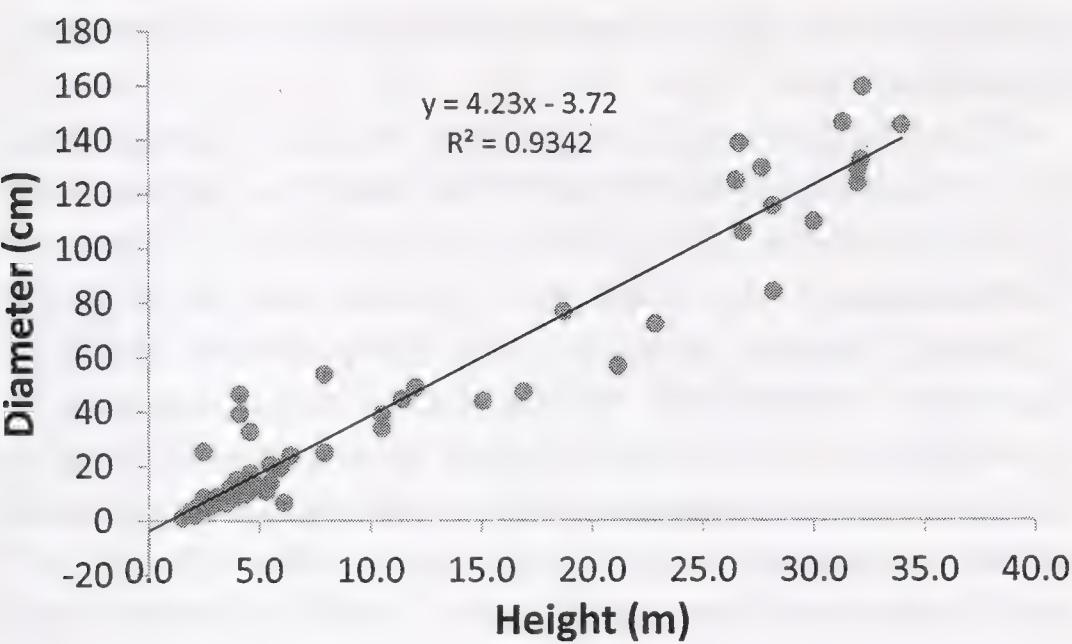


FIG. 4. Relationship of diameter at breast height to height of *Pinus monticola* growing in wet meadow and mesic bench habitats in the Emerald Lake watershed (n = 65).

a mean basal area of 0.3 m² ha⁻¹. The conifer community here was dominated by western white pine, with a significant presence of lodgepole pine. The steep talus slopes, the smallest of the habitat areas, was home to only 4.7% (57 trees) of the watershed trees, and 1.0% (1.7 m²) of basal area. Mean tree density, however, was 17.6 trees ha⁻¹ but with only a mean basal area of 0.5 m² ha⁻¹ because of small tree sizes. Almost all of the trees on these slopes were western white pine.

Diameter at breast height showed a linear relationship to tree height in western white pine (Fig. 4). Seven trees in those sampled had heights >30 m, with the tallest reaching 34 m. Several trees with intermediate diameters of 40–80 cm had heights well below that predicted for a regression, reflecting a combination of poor growing sites in granite cracks or past lightning strikes.

Tree Growth Rates and Needle Retention

The timing of the phenological events of new needle initiation, needle maturity, and branch elongation of growing tips varied among years for lodgepole pine and western white pine. Juvenile needles began to form in late May and continued to expand until early August. Branch elongation was apparent in up to 50% of trees by late June and was completed by early August, reflecting a short growing season in this habitat. Preformed buds for the following growing season were visible beginning

TABLE 4. Relative needle retention for 5 yr in *Pinus balfouriana*, *P. contorta* subsp. *murrayana*, and *P. monticola* in the Emerald Lake watershed. Values shown are percent needle retention at the end of each growing season from the cohort of needles formed in 1982.

Year	<i>P. balfouriana</i>	<i>P. contorta</i>	<i>P. monticola</i>
1 (1982)	100	98	100
2 (1983)	91	95	91
3 (1984)	83	81	83
4 (1985)	80	79	68
5 (1986)	64	67	42

in early to late August depending on the year. Mean needle length, annual twig growth, and biomass per annual growth node showed variation, but no consistent pattern over the 5 yr of study for the three dominant pine species (Table 3).

Relative needle retention over the 5 yr of study was very similar in the three dominant in species, with over 81–83% retention of first growing season cohorts of needles at the end of the third growing season (Table 4). By the end of five growing seasons, western white pine retained only 40% of its of 1982 growing season cohorts of needles, while foxtail pine and lodgepole pine retained close to 64%–67%, respectively.

DISCUSSION

Subalpine stands of conifers in the Emerald Lake watershed are representative of those in other parts of the central and southern Sierra Nevada (Millar and Rundel 2016). Western white pine, the dominant species at Emerald Lake, is locally abundant in subalpine habitats along the western slope of the Sierra Nevada, where it may occur in small pure stands but more commonly is found mixed with lodgepole pine, Jeffrey pine, mountain hemlock, and red fir (Potter 1998). Although trees of this species may reach 40 m in height and 2.5 m in diameter in the Sierra Nevada, these sizes are smaller than those reached by western white pines in the northern Rocky Mountains and Pacific Northwest (Van Pelt 2001). Western white pine generally maintains a tree form of growth up nearly to treeline, where it is commonly replaced in the Sierra Nevada by white-

TABLE 3. Five-year record of mean needle length, twig length for new growth, and biomass per growing tip for *Pinus balfouriana* (PIBA), *Pinus contorta* subsp. *murrayana* (PICO), and *Pinus monticola* (PIMO) in the Emerald Lake watershed, Sequoia National Park. Field samples were collected in October and November 1986. Standard deviations of mean values for measurements of 20 branches collected from five trees are shown in parentheses.

	Needle length (mm)			Twig length (mm)			Biomass / node (mg)		
	PIBA	PICO	PIMO	PIBA	PICO	PIMO	PIBA	PICO	PIMO
1986	24 (5)	33 (7)	41 (6)	13.8 (6.2)	21.6 (7.0)	22.2 (9.4)	799 (539)	1090 (418)	970 (328)
1985	25 (4)	46 (8)	49 (4)	16.9 (6.1)	30.4 (9.8)	25.9 (8.5)	962 (546)	2271 (742)	1428 (399)
1984	25 (4)	39 (6)	47 (5)	14.1 (5.1)	20.6 (4.1)	21.1 (8.6)	929 (429)	1463 (380)	1087 (300)
1983	18 (5)	25 (6)	30 (8)	12.1 (5.0)	20.6 (5.2)	19.4 (7.0)	744 (640)	937 (386)	698 (236)
1982	21 (5)	33 (9)	41 (5)	12.3 (4.1)	21.0 (5.4)	21.6 (11.1)	717 (507)	1217 (461)	885 (359)

bark pine or foxtail pine on rocky ridges (Millar and Rundel 2016). Western white pine seedlings are reported to be relatively rare compared to other subalpine conifers (Parker 1988).

Open stands of lodgepole pine forests make up a widespread upper montane forest/woodland over much of the species' range, tolerating both shallow rocky soils and semi-saturated meadow edges, in an elevational belt both within and above the red fir zone (Potter 1998; Fites-Kaufman et al. 2007). These open forests generally occur at elevations of about 1,830–2,400 m in the northern Sierra Nevada, and rise to 2440–3350 m in the south. Thus, the Emerald Lake basin lies near the upper elevational limits of this species. Topography has a strong influence on elevational distribution, and lodgepole pine forests may reach into much lower elevations with cold air drainage down glacial canyons (Potter 1998; Fites-Kaufman et al. 2007).

Foxtail pine is the dominant treeline pine in the southern Sierra Nevada, where it is restricted to higher elevations of 2,600–3,660 m south of the Middle Fork of the Kings River. At its lower elevational limits, this species may occur in open stands with lodgepole pine, Jeffrey pine, western white pine, and red fir (Millar and Rundel 2016). At higher elevations, however, it forms relatively pure, but low density stands. It has been suggested that foxtail pine populations in the southern Sierra Nevada likely persisted through the Pleistocene in ice-free ridgetop habitats (Eckert et al. 2008). Field studies have suggested that soil moisture availability is the most critical variable for determining fine-scale patterns of growth (Bunn et al. 2005).

Vankat and Major (1978) sampled stands of foxtail pine from elevations of 3170–3290 m in Sequoia National Park and reported a relatively high mean density of 418 tree ha⁻¹, with a canopy cover of 26%, and a basal area of 31 m² ha⁻¹. The present study found much lower densities, but higher basal area. Tree densities and stand basal areas decline with increasing elevation from foxtail pine woodlands to treeline (Lloyd 1997). Foxtail pines have great longevity and can grow to be several thousand years old (Millar and Rundel 2016). Like bristlecone pine, foxtail pine has wood that is highly resinous, and, combined with cold, arid climates in the southern Sierra Nevada, remnant dead wood can persist for millennia. Together the live and dead wood are important archives for paleoclimatic and paleoecologic study, and foxtail pines have been documented to respond to warm and cold historic climate periods by, respectively, advancing upslope and retracting downslope (Scuderi 1993; Lloyd and Graumlich 1997; Millar et al. 2004).

Jeffrey pine commonly occurs on rocky or more arid slopes at elevations of 1600–2600 m in the southern Sierra Nevada (Millar and Rundel 2016), but reaches to 3000 m or more in favorable sites as at Emerald Lake. Similarly, the small population of red fir at Emerald Lake occurs near the upper elevation

limit of this species and is restricted to the most favorable sites.

The results from elongation rates of pine branch tips as a surrogate for growth conditions suggest that interannual variation in these conditions have not been dramatically different over the period of years sampled. Increasing impacts of air pollution on high elevation watersheds in California were one of the concerns that led to the broad program of Emerald Lake studies (Tonnessen 1991). Shortened periods of needle retention, a measure associated with oxidant air pollution (Arbaugh et al. 2003), were not apparent in the current study.

Historical studies of the growth dynamics of subalpine trees in the Sierra Nevada have highlighted the complexity of responses to cyclic interactions between temperature and moisture at a regional level (Millar et al. 2004; Millar and Rundel 2016). Spatial tree patterns in these ecosystems (exemplified here by the Emerald Lake watershed) respond directionally to century-long climate trends, and may exhibit abrupt and reversible effects as a consequence of multi-decadal climate variability. Significant interactions among temperature, multiyear variability in moisture availability, and the Pacific Decadal Oscillation are all interrelated factors, with ecological responses driven in particular by minimum temperatures and their impact on snowmelt (Millar and Rundel 2016). Thus, global change may well have very strong impacts on the structure of subalpine tree communities, although with some expected lag time in these long-lived species. The detailed population structure of conifers collected three decades ago in the Emerald Lake watershed may serve as a basis for interpreting possible future changes in stand structure and growth rates.

There is growing evidence that rates of warming are elevation-dependent and that higher elevations are changing faster than lower elevations, perhaps due to increased sensitivity to forcing factors (Diaz and Bradley 1997). If this is the case, and exceptional growth rates for subalpine species (e.g., bristlecone pine in the White Mountains, Salzer et al. 2009), suggest that it is, then subalpine forests might well face rates of warming faster than projected for the California lowlands. Temperature may no longer be the main limiting factor for growth of bristlecone pine on south-facing slopes near treeline in the White Mountains, suggesting that increasing warmth may lead to a divergence between tree growth and temperature at previously temperature-limited sites (Salzer et al. 2014).

Mountains have enormous heterogeneity in slope, aspect, topography, regional context, and landform, as seen in the distinct, but separate, community assemblages of conifers within the Emerald Lake watershed. This patchiness translates to complexity in meso- and micro-climatic variation (Daly et al. 2007; Lundquist and Cayan 2007; Graham et al. 2012), potentially providing subalpine and alpine plants and animals with potential favorable escape

opportunities other than simple migration upslope. An example of this can be seen where cold-air drainage creates positive lapse rate conditions (i.e., decreasing temperature with declining elevation) in montane environments. There are hints that this process might actually increase in the future under forcing factors of anthropogenic climate change (Pepin and Lundquist 2008), resulting in the net effect that canyon bottoms, basins, and swales may become cooler than slopes and summits. In addition, diverse microclimates relatively independent of elevation may occur as with special geomorphic features, such as rock glaciers, buffer local conditions of temperature and moisture availability (Millar and Rundel 2016; Millar et al. 2016).

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THE IDENTITY OF *TRENTEPOHLIA* ALGAE (CHLOROPHYTA: TRENTEPOHLIALES)
FROM POINT LOBOS STATE RESERVE AND THE SAN FRANCISCO REGION,
CALIFORNIA

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ABSTRACT

Terrestrial chlorophyte algae of the order Trentepohliales are found in humid habitats around the world. Although most diverse and abundant in the tropics, on a global scale the group is nearly cosmopolitan. The most distinctive macroscopic trait of the Trentepohliales is their orange, golden, or red color, which is due to the accumulation of carotenoid pigments. Orange algae of the genus *Trentepohlia*, the most species-rich genus in the order, can be found growing on trees, rocks, and other substrates along the Pacific Coast of the United States, including the San Francisco Bay region. Perhaps the best known site for these algae is at Point Lobos State Reserve in Carmel, CA. The orange algae at this site are often referred to by California naturalists as “*Trentepohlia aurea*” or “*Trentepohlia aurea* var. *polycarpa*”. Given the challenges in identifying species in this genus and some confusion about nomenclature, we sampled *Trentepohlia* at Point Lobos and elsewhere in the region to clarify the identity of these algae. We confirm that the *Trentepohlia* at Point Lobos (and most of the *Trentepohlia* we sampled at other sites) are *T. flava*, which was long considered to be a form of *T. aurea* and was often reported under the heterotypic synonym *T. aurea* var. *polycarpa*. *Trentepohlia abietina* was the only other species recorded and our observations suggest the possibility that in this region the two species may exhibit different substrate preferences (*T. flava* generalist on many different substrates, *T. abietina* limited to tree bark).

Key Words: *Trentepohlia*, *Trentepohlia abietina*, *Trentepohlia abietina* var. *corrugata*, *Trentepohlia aurea*, *Trentepohlia aurea* var. *polycarpa*, *Trentepohlia flava*, *Trentepohliales*.

The order Trentepohliales is a group of terrestrial green algae widespread in habitats characterized by high atmospheric humidity (Printz 1939; Chapman 1984). These algae are most diverse and abundant in humid tropical regions, where the majority of the species occur. On a global scale, however, their distribution is nearly cosmopolitan. In recent decades, these algae have drawn the attention of specialists due to several intriguing ultrastructural features (Chapman and Henk 1986; López-Bautista et al. 2002) and their peculiar evolutionary status as a highly diverse terrestrial group in a class (Ulvophyceae) composed mainly of marine benthic algae (López-Bautista and Chapman 2003). Members of this group, however, were already known to early naturalists of the 18th century, who provided the first species descriptions (e.g., Linnaeus 1753; Wiggers 1780). The most distinctive macroscopic trait of the Trentepohliales is their orange, golden, or red color, which is due to the accumulation of carotenoid pigments (particularly β -carotene). Members of this group can be recognized as brightly colored patches

or tufts growing on the surface of many aerial substrates, such as rocks, tree bark, leaves, twigs, fruits, soil, woodwork, and concrete. They are usually most conspicuous when growing in sun-exposed habitats, where the high irradiance stimulates the production of carotenoids.

At present, the order Trentepohliales includes approximately 115 species (Guiry and Guiry 2017), classified in the genera *Cephaleuros* Kunze, *Phycopeltis* Millardet, *Printzina* Thompson & Wujek, *Stomatochroon* Palm, and *Trentepohlia* Martius. The presence of Trentepohliales in the United States has been long known, particularly in the warm and humid southeastern states, where species of the genus *Cephaleuros* grow epiphytically or endophytically on or in the leaves of many vascular plants (Brooks et al. 2015). However, Trentepohliales are not restricted to these regions, and populations of these algae also commonly occur in many humid maritime areas of the Pacific Coast. In this case, the genus involved is *Trentepohlia*, the earliest-known and most species-rich genus in the order. *Trentepohlia* algae are



FIG. 1. Appearance of *Trentepohlia* in field populations sampled along the California coast. Top left: *T. flava* on rock (Point Lobos State Reserve). Top right: *T. flava* on cypress (Land's End). Middle left: *T. flava* on rock (Point Lobos State Reserve). Middle right: *T. flava* on dead cypress (Point Lobos State Reserve). Bottom left: *T. abietina* on tree (Presidio). Bottom right: *T. abietina* var. *corrugata* and *T. flava* on cypress tree (Great Highway). Photos by James Sikes.

composed of uniseriate filaments variously branched and joined to form dense tufts or thin crusts. Species of this genus reproduce sexually with the production of biflagellate gametes in globular or egg-shaped gametangia; asexual reproduction involves quadri-flagellate zoospores produced in globular or egg-shaped sporangia borne at the top of a cell with a characteristic retrorsely bent neck (suffultory cell). The main taxonomic characters used for morphological identification of *Trentepohlia* species are shape and size of cells, branching pattern, presence/absence of hair-like cells, and position and habit of gametangia and zoosporangia. However, it is known that some of these features may vary under different environmental conditions, making species delimitation sometimes uncertain. For this reason *Trentepohlia* is considered a taxonomically complex genus, in which morphological identification can be difficult (Rindi and Guiry 2002).

Many naturalists in California are familiar with a conspicuous orange-colored green alga seen growing on aerial substrates, particularly the bark of trees (most famously on Monterey cypress, *Hesperocyparis macrocarpa* [Hartw.] Bartel). Such growths are common in the whole coastal area of northern and central California, but are particularly well developed at certain sites, such as Point Lobos State

Reserve (Fig. 1). These algae are sometimes referred to as *Trentepohlia aurea* var. *polycarpa*, presumably referring to *Trentepohlia aurea* var. *polycarpa* (Nees and Montagne) Hariot, but most sources refer to them simply as *Trentepohlia aurea*, implying *Trentepohlia aurea* (Linnaeus) Martius. We became curious about the validity of these identifications and made field collections with the goal of clarifying the identity of this widespread alga.

METHODS

We obtained 39 samples of *Trentepohlia* from seven different sites in the San Francisco region (Table 1). Most samples were collected from Monterey cypress bark, where the most obvious growths were observed, but they were also found on other substrates (Table 1). The samples were allowed to air dry indoors before being placed in Ziploc bags and mailed to Università Politecnica delle Marche, where they were processed by Fabio Rindi (Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Italy). The samples were examined by light microscopy and identified based on morphological features, using the main taxonomic treatments for the genus *Trentepohlia* (Hariot 1889; Printz 1939; Cribb 1970; Sarma 1986). Voucher

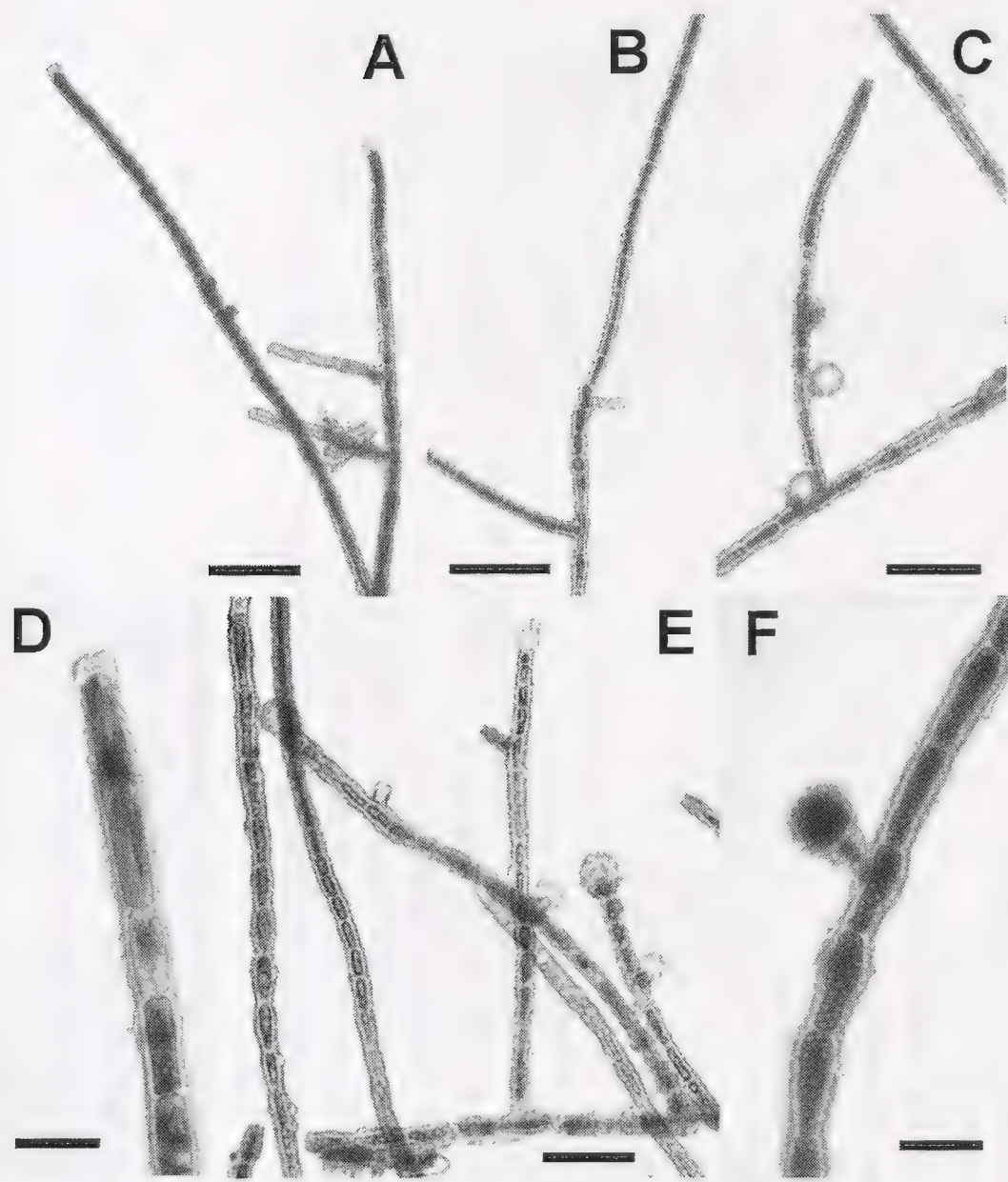


FIG. 2. Morphology of *Trentepohlia flava*. A. habit of erect axes; scale bar: 100 μ m. B. habit of an erect axis with cells of various shapes and sizes; scale bar: 150 μ m. C. habit of erect axes bearing some empty gametangia; scale bar: 100 μ m. D. apex of erect axis showing apical cell with pectic cap; scale bar: 30 μ m. E. habit of erect axes bearing empty gametangia; scale bar: 100 μ m. F. detail of a gametangium borne on a short lateral branch, with heavily corrugated surface; scale bar: 30 μ m. Photos by Fabio Rindi.

specimens are conserved in the personal herbarium of Fabio Rindi.

RESULTS

Microscopic observation revealed that the samples belonged to two different species: *Trentepohlia flava* (Kützinger) De Toni and *T. abietina* (Flotow) Hansgirg. The distinction between these species was unambiguous and no uncertainties in their identifications were found. The two species were readily distinguished based on the size of the erect axes, width of the cells, and corrugation of the cell walls.

Trentepohlia flava was the dominant species, being recorded in 36 of the 39 samples; in 35 samples it was the only species present, whereas in one sample it was mixed with *T. abietina* var. *corrugata* (Leighton) Cribb. All samples from Point Lobos State Reserve included only *T. flava*. *Trentepohlia flava* occurred on tree bark, but also on wooden fences and rocks. Specimens of *T. flava* consisted of erect axes up to 1.5 mm tall, unbranched to sparsely branched with irregular or unilateral pattern, arising from a limited base of prostrate axes (Fig. 2A, B, C). Cells were cylindrical or slightly swollen, 15–20 (up to 25) μ m wide and 1.5–6 times as long as wide, with a thick and heavily corrugated cell wall. A well-developed

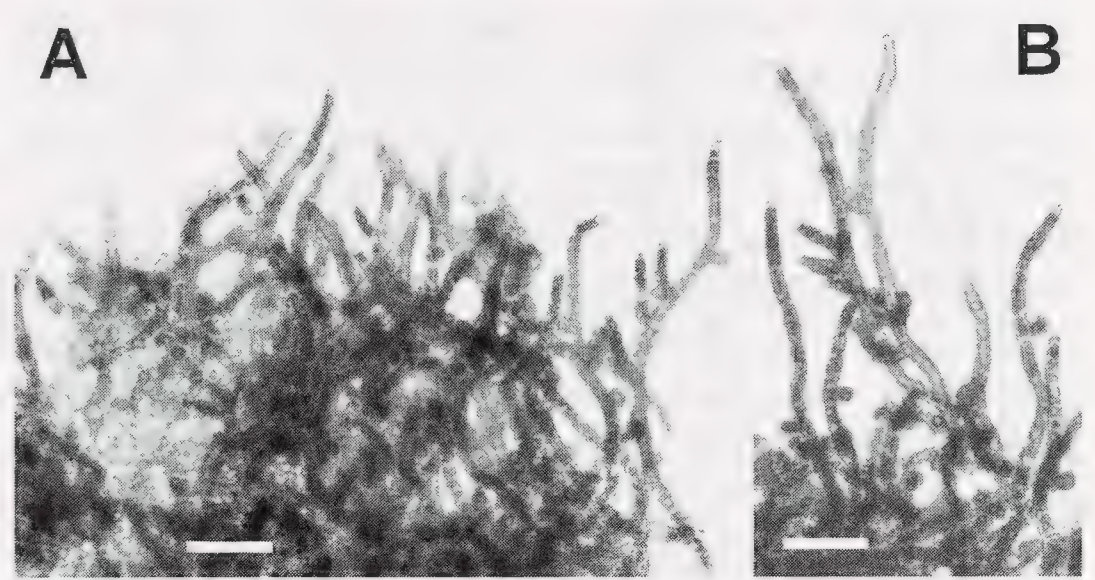


FIG. 3. Morphology of *Trentepohlia abietina*. A. habit; scale bar: 50 μ m. B. detail of some erect axes; scale bar: 50 μ m. Photos by Fabio Rindi.

pectic cap occurred at the top of each apical cell (Fig. 2D). Gametangia were present in the majority of the samples of *T. flava*; these were subglobular to egg-shaped, 25–36 (up to 40) μ m wide, lateral or apical on the erect axes or apical at the top of short lateral branches (Fig. 2C, E, F). Their surface was also heavily corrugated (Fig. 2F). Release of gametes was not observed.

Trentepohlia abietina was found in four samples. Three of these (Presidio 1, 2, and 3), all growing on tree bark, were identified as the nominate form of *T. abietina*, whereas a fourth, identified as *T. abietina* var. *corrugata*, was collected from the bark of a cypress tree beside the Great Highway (Table 1). *Trentepohlia abietina* formed thin turfs consisting of numerous interwoven specimens (Fig. 3A); each specimen consisted of one or more erect axes, unbranched or irregularly branched, up to 500 μ m tall and arising from a limited prostrate portion. Cells were cylindrical or swollen, 7.5–10 μ m wide (Fig. 3B); small pectic caps occurred at the top of many apical cells. Gametangia were abundant, globular in shape, and 15–20 μ m in diameter. In individuals from the Great Highway, the width of the cells was higher (12–16 μ m) and the cell walls were moderately corrugated; based on these characteristics, these specimens were identified as *T. abietina* var. *corrugata*.

After examination of our samples, we conclude that *T. flava* is the dominant species of *Trentepohlia* at coastal sites in the San Francisco region. *Trentepohlia abietina* is also present but, based on our limited sampling, does not appear to be as abundant as *T. flava*. Our results also suggest a possible ecological differentiation between the two species, with *T. flava* able to grow on a wide range of living and nonliving substrates, but *T. abietina* possibly limited to tree bark.

DISCUSSION

Trentepohlia flava is a well-defined species, whose taxonomic circumscription is now well established from the morphological point of view. It was originally described as *Mycinema? flava* by Hooker

TABLE 1. *TRENTEPOHLIA* SAMPLING IN CALIFORNIA.

Sample ID	Date	Site name	Location	Latitude	Longitude	Substrate	Species ID
17-1	22-Apr-17	Fort Funston	San Francisco	37.7202	-122.5043	cypress tree along trail on sandy flat adjacent to Pacific Ocean	<i>T. flava</i>
17-2	22-Apr-17	Fort Funston	San Francisco	37.7213	-122.5037	cypress tree along trail on sandy flat adjacent to Pacific Ocean	<i>T. flava</i>
17-3	22-Apr-17	Great Highway	San Francisco	37.7615	-122.5098	cypress tree along highway where urban development meets Pacific Ocean	<i>T. abietina</i> var.
17-4	22-Apr-17	Lands End	San Francisco	37.7841	-122.5086	cypress tree along trail along rocky outcrop ~125 m from Pacific Ocean	<i>corrugata</i> & <i>T. flava</i>
17-5	22-Apr-17	Lands End	San Francisco	37.7845	-122.5088	cypress tree along trail along rocky outcrop ~150 m from Pacific Ocean	<i>T. flava</i>
17-6	14-Apr-17	Point Lobos	Carmel	36.5215	-121.9512	cypress tree along trail along rocky outcrop adjacent to Pacific Ocean	<i>T. flava</i>
17-7	14-Apr-17	Point Lobos	Carmel	36.5221	-121.9512	cypress branch	<i>T. flava</i>
17-8	14-Apr-17	Point Lobos	Carmel	36.5221	-121.9536	cypress branch	<i>T. flava</i>
17-9	14-Apr-17	Point Lobos	Carmel	36.5227	-121.9520	cypress base branches	<i>T. flava</i>
17-10	14-Apr-17	Point Lobos	Carmel	36.5221	-121.9532	cypress trunk	<i>T. flava</i>
17-11	14-Apr-17	Point Lobos	Carmel	36.5228	-121.9522	dead cypress trunk	<i>T. flava</i>
17-12	14-Apr-17	Point Lobos	Carmel	36.5221	-121.9536	cypress branch exposed to ocean	<i>T. flava</i>
17-13	14-Apr-17	Point Lobos	Carmel	36.5220	-121.9533	dead cypress branch	<i>T. flava</i>
17-14	14-Apr-17	Point Lobos	Carmel	36.5224	-121.9523	cypress branches	<i>T. flava</i>
17-15	14-Apr-17	Point Lobos	Carmel	36.5219	-121.9533	dead cypress branches	<i>T. flava</i>
17-16	14-Apr-17	Point Lobos	Carmel	36.5219	-121.9533	cypress branches	<i>T. flava</i>
17-17	14-Apr-17	Point Lobos	Carmel	36.5219	-121.9537	seaward facing rock	<i>T. flava</i>
17-18	14-Apr-17	Point Lobos	Carmel	36.5222	-121.9530	dead exposed roots	<i>T. flava</i>
17-19	14-Apr-17	Point Lobos	Carmel	36.5220	-121.9533	rock	<i>T. flava</i>
17-20	14-Apr-17	Point Lobos	Carmel	36.5219	-121.9534	cypress branch	<i>T. flava</i>
17-21	14-Apr-17	Point Lobos	Carmel	36.5220	-121.9533	rock	<i>T. flava</i>
17-22	14-Apr-17	Point Lobos	Carmel	36.5222	-121.9537	rock	<i>T. flava</i>
17-23	14-Apr-17	Point Lobos	Carmel	36.5221	-121.9532	cypress tree trunk	<i>T. flava</i>
17-24	14-Apr-17	Point Lobos	Carmel	36.5226	-121.9518	cypress branch	<i>T. flava</i>
17-25	14-Apr-17	Point Lobos	Carmel	35.5221	-121.9527	cypress branch	<i>T. flava</i>
17-26	14-Apr-17	Point Lobos	Carmel	35.5219	-121.9533	cypress branch	<i>T. flava</i>
17-27	22-Apr-17	Presidio 1 (Lake & 12th)	San Francisco	37.7874	-122.4704	tree near lake in urban park ~1400 m from Pacific Ocean	<i>T. abietina</i>
17-28	22-Apr-17	Presidio 2 (1st Dr. E)	San Francisco	37.7995	-122.4631	tree in urban development adjacent to cemetery ~600 m from SF Bay	<i>T. abietina</i>
17-29	22-Apr-17	Presidio 3 (Moraga Ave)	San Francisco	37.7976	-122.4583	tree in urban development ~800 m from SF Bay	<i>T. abietina</i>
17-30	2-May-17	Sharp Point	Pacifica	37.6205	-122.4964	cypress tree along trail along rocky outcrop adjacent to Pacific Ocean	<i>T. flava</i>
17-31	22-Apr-17	Sutro Heights	San Francisco	37.7775	-122.5122	cypress tree along trail along rocky outcrop in urban area adjacent to Pacific Ocean	<i>T. flava</i>

TABLE 1. CONTINUED

Sample ID	Date	Site name	Location	Latitude	Longitude	Substrate	Species ID
17-32	22-Apr-17	Sutro Heights	San Francisco	37.7776	-122.5122	cypress branch	<i>T. flava</i>
17-33	22-Apr-17	Sutro Heights	San Francisco	37.7776	-122.5122	cypress branch	<i>T. flava</i>
17-34	22-Apr-17	Sutro Heights	San Francisco	37.7776	-122.5123	wooden fence (unknown wood type)	<i>T. flava</i>
17-35	22-Apr-17	Sutro Heights	San Francisco	37.7777	-122.5124	cypress branch	<i>T. flava</i>
17-36	22-Apr-17	Sutro Heights	San Francisco	37.7775	-122.5122	cypress trunk/large branch	<i>T. flava</i>
17-37	22-Apr-17	Sutro Heights	San Francisco	37.7777	-122.5124	branch	<i>T. flava</i>
17-38	22-Apr-17	Sutro Heights	San Francisco	37.7777	-122.5124	branch	<i>T. flava</i>
17-39	22-Apr-17	Sutro Heights	San Francisco	37.7777	-122.5114	fallen branch	<i>T. flava</i>

and Arnott (1832) based on material collected in Concepcion, Chile, from leaves of *Quadria heterophylla* Ruiz & Pavón (now *Gevuina avellana* Molina). Since Hooker and Arnott (1832) were unsure of the placement at the genus level, *Mycinema? flava* is a taxonomically invalid name and the name of the species was nomenclaturally validated by Kützinger (1843) as *Chroolepus flavum*, the basionym of *T. flava* (Kützinger) De Toni. Cribb (1970) provided a detailed morphological circumscription of *T. flava* based on examination of the type specimen of *Mycinema? flava* and other important herbarium specimens.

Rindi et al. (2009) sequenced the *rbcL* gene in specimens identified by them as *T. flava*, collected from a coastal area of the Atacama Desert, Chile, and from Inverness, Tomales Bay, California (a site located about 200 km up the coast from Point Lobos). Despite the great distance between Chile and California, these specimens had almost identical *rbcL* sequences, strongly supporting the conspecificity of the two populations. This suggests that *T. flava* is a widespread species that has a continuous distribution along the Pacific shores of the Americas. Our specimens from Point Lobos are morphologically identical to both the Chilean and Californian samples sequenced by Rindi et al. (2009) and agree well with the morphological circumscription given by Cribb (1970). Thus, our identification of *Trentepohlia flava* from Point Lobos is strongly supported by morphological evidence (which indirectly suggests also a likely molecular identity of *rbcL* sequence with the material from Inverness and Atacama). The thick, heavily corrugated cell walls and the tall erect axes (unbranched to sparsely branched) make *T. flava* a morphologically distinct alga; these features are very consistent in this species and were also observed in specimens from the Oregon coast (Rindi unpublished data) and southern Africa (Rindi et al. 2006).

Trentepohlia aurea, a name often applied to the Point Lobos algae, is the type species of the genus and was originally described by Linnaeus (1753) as *Byssus aurea* based on collections from Europe (Wales and Italy). The morphological circumscription of this species has been the subject of some debate and numerous subspecific taxa have been erected for it (see Guiry and Guiry 2017). For some time, the distinction of *T. aurea* and *T. flava* was questioned by some authors (Hariot 1889; Printz 1939) who did not separate these entities at the species level; they considered *T. flava* a variety of *T. aurea*, reporting it under the heterotypic synonym *T. aurea* var. *polycarpa* (based on *Trentepohlia polycarpa* Nees & Montagne, now considered a heterotypic synonym of *T. flava*). Cribb (1970), after examination of the type of *Mycinema? flava* and other herbarium specimens, concluded that the two entities are different species, arguing that the wall ornamentation of *T. flava* is a stable character distinguishing it from *T. aurea*. Based on our experience, we agree with Cribb (1970); in the past, one of us (F. Rindi) has examined numerous

specimens of *T. aurea* from Europe, all of which differed substantially from *T. flava* and never showed heavily corrugated walls. Some of these specimens were sequenced in molecular phylogenetic studies (López-Bautista et al. 2006; Rindi et al. 2009) and molecular data supported the species level separation of *T. aurea* and *T. flava*. In molecular phylogenies including samples of both species (Rindi et al. 2009; Nelsen et al. 2011), *T. aurea* forms a strongly supported clade well separated from *T. flava*. During our survey we could not find any specimens morphologically referable to *T. aurea* and we conclude that, at present, the occurrence of this species in the San Francisco region cannot be confirmed.

We refer to *Trentepohlia abietina* the three samples collected at Presidio (1, 2, 3) and to its variety *corrugata*, the sample from Great Highway. *Trentepohlia abietina* was originally described by Kützinger (1845) using material collected from the bark of fir trees (*Abies alba* Miller) at Hirschberg (probably Hirschberg im Riesengebirge, currently Jelenia Góra, southern Poland). The morphological circumscription of this species is generally straightforward and records of it are available for temperate and tropical regions throughout the world (Printz 1939; Sarma 1986; Rindi and Guiry 2002; Rindi et al. 2006). It should be noted, however, that reliable molecular data for this species are scant and currently insufficient for an accurate phylogenetic assessment. López-Bautista et al. (2006) sequenced the small subunit ribosomal RNA region in samples of *T. abietina* from Ireland, which indicated a close relationship with *T. umbrina* (Kützinger) Bornet, *T. jolithus* (Linnaeus) Wallroth, and *Phycopeltis arundinacea* (Montagne) De Toni. However, in subsequent studies other samples morphologically similar to *T. abietina* were recovered in different positions in molecular phylogenies (*rbcL* phylogenies reported by Rindi et al. [2009] and Nelsen et al. [2011]). This suggests the possibility that the morphology of *T. abietina* may be shared by several cryptic species not closely related from a phylogenetic point of view. This would pose a major problem for the correct identification of the species and the correct application of the name *T. abietina*. This issue can be resolved only with a large-scale reassessment of the species, based on a molecular dataset including sequences of specimens from the type locality, Hirschberg, and many other locations covering the whole geographical range of the species. Since such data are currently not available, we base the identification of our specimens on the traditional morphological circumscription of the species established by previous authors (De Toni 1889; Hariot 1889; Printz 1939).

In conclusion, we confirm that specimens from coastal areas of California previously reported as *T. aurea* var. *polycarpa* were correctly identified. However, this alga is no longer considered a variety of *T. aurea* and we recommend that these specimens

should be referred to as *T. flava*, which is presently the accepted name for this alga.

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COMMON PERSIMMON (*DIOSPYROS VIRGINIANA* L.), A NATURALIZED, POTENTIALLY INVASIVE SPECIES IN THE STATE OF CALIFORNIA

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ABSTRACT

Common persimmon (*Diospyros virginiana* L.) is a deciduous tree occurring widely in eastern North America, as far west as Texas. The species is known for producing relatively large, edible fruits that were consumed by Native Americans and by the European colonists who arrived in North America more recently. Because of its fruit production, *D. virginiana* is broadly planted outside its natural range. Here, I report on five naturalized occurrences of *D. virginiana* found in the state of California, where the species is not native. In 2016 and 2017, these populations were surveyed and monitored for fruit production. In all populations, mature fruits were found in the autumn, and these fruits were observed to produce viable seeds with filled embryos. Two of the populations have greater than 70 individuals and contain trees at varying levels of maturity. It is not yet known if these populations are the result of asexual reproduction via root sprouting or sexual reproduction via diaspores. However, the production of viable diaspores in all surveyed populations indicates that sexual seed set is occurring. The large size of two stands, one along a creek in Butte County and the other on a river flood plain in Tulare County, suggests that some areas of California might be susceptible to invasion by this species. Future research should try to determine whether recruitment via diaspores is occurring, and whether these seeds are the product of selfing in perfect-flowered plants, or cross fertilization in a population containing both male and female plants, as this would have important implications for the potential invasiveness of this species in the state.

Key Words: Butte County, California, Tulare County, persimmon, riparian.

Common persimmon (*Diospyros virginiana* L. var. *virginiana*, Ebenaceae) is a medium sized deciduous, dioecious tree, reproducing sexually via seeds embedded in fleshy berries and vegetatively by resprouting from roots and the buried root crown (Eckenwalder 2009). *Diospyros virginiana* is a widespread element of temperate deciduous forests in the eastern United States, with its native range spanning from Connecticut south into northern Florida, and as far west as eastern Texas (Eckenwalder 2009). The plant has long been appreciated for its edible fruits, consumed by indigenous people of what became the United States, and then by European colonists and their descendants (Briand 2005). The plant has a long history of cultivation, including by indigenous people prior to the arrival of European colonists, and is still grown for its fruits in some areas of the United States (Briand 2005). Although the species does not occur natively west of Texas (Eckenwalder 2009), it has been documented in California as a cultivated waif (Hrusa et al. 2002; Baldwin et al. 2012; Baldwin, University of California, Berkeley personal communication), and also as a naturalized part of the introduced flora (Jepson Flora Project 2017). Nevertheless, the species is not yet included as naturalized in the most authoritative reference manual of California vascular plants, the Jepson eFlora (Jepson Flora Project 2017).

As discussed by Hrusa et al. (2002), the fact that this plant has been cultivated for hundreds of years in the United States (Briand 2005) and is capable of persisting for some time as a mature tree, may compromise our ability to determine whether existing

trees represent population recruitment or are simply the survivors of historical orchard plantings. As with many cultivated plants, this ambiguity is why this plant has not been widely recognized as naturalized in the state (Baldwin, University of California, Berkeley personal communication). In addition to a lack of consensus as to the ability of this plant to persist under natural conditions, nothing is known about the potential for this tree to become invasive (*sensu* Richardson et al. 2000) in the state.

In 2015, the author discovered a large, but previously unreported (Oswald and Ahart 1998), population of *D. virginiana* in the lower Butte Creek watershed, Butte County, CA (Table 1). Discovery of this extensive and seemingly expanding population (Burge personal observation) made the author wonder if this fast-growing, non-native might pose a risk as an invasive species. Inspired by the Butte County discovery, the author visited several other reported occurrences of *D. virginiana* in California. Data were collected on population size, age structure, and capacity for sexual reproduction. The overall aim of this work was to determine 1) whether this plant is naturalized in the state of California, and thus a candidate for inclusion in the Jepson Flora Project, and 2) if there is any risk that this tree might become an invasive species in the state.

METHODS

In the autumn and early winter of 2016, the author visited six known populations (i.e., occurrences) of

TABLE 1. SURVEYED SITES, WITH DEMOGRAPHIC AND SEED VIABILITY DATA. Site code corresponds to the collecting code used by the author (Appendix 1). Viable seed refers to whether the seeds collected at a site had filled embryos (based on a cut test).

Site code	County	Viable seed present?	Demographics					
			Adult diameter at breast height (cm)			Sapling height (m)		
			n	Range	Average \pm SD	n	Range	Average \pm SD
2184	Butte	Yes	66	2.1–37.5	8.5 \pm 6.7	8	0.72–1.54	1.19 \pm 0.31
2202	Tulare	Yes	76	2.2–33.4	8.5 \pm 6.1	31	0.60–1.25	1.50 \pm 0.39
2210	San Bernardino	Yes	9	2.2–10.5	7.2 \pm 3.0	0	–	–
2211	San Bernardino	Yes	10	2.0–42.0	8.0 \pm 12.1	15	0.5–2.2	1.30 \pm 0.60
2215	San Diego	Yes	10	4.7–27.4	14.9 \pm 6.3	0	–	–
2217	San Diego	No	1	8.3	–	0	–	–

D. virginiana, in addition to the one in Butte County discovered by the author (Table 1). These additional occurrences were selected from among the records of Ebenaceae catalogued by 1) the Consortium of California Herbaria (CCH 2017), 2) the Biota of North America Program (BONAP) (Kartesz et al. 2017), and 3) Hrusa et al. (2002). Occurrences were selected on the basis of their likelihood to represent naturalized occurrences.

Occurrences were accessed via vehicle and on foot. At each occurrence, the presence of live plants was verified, and a voucher specimen was collected. In each population, the author made a survey on foot, attempting to access every tree in the population. The author took a measurement of diameter at breast height (dbh) for all observed individuals over one cm dbh and measured the height of all other trees (seedlings and saplings). In all the populations, other than the Kaweah River site (D. Burge 2202, Table 1), every plant in the local population was measured. At the very large Kaweah River population, an area of 10,000 m² was surveyed (100 m \times 100 m) to provide a snapshot of the demographics of the plants found in this area (Appendix 1). All dbh measurements were made using a flexible, metric measuring tape; all measurements of height were made with a metric scale construction tape measure. The author also surveyed for the presence of ripe fruits and collected whole seeds from fruits when present. Whole seeds were cleaned of flesh under running water and air dried. The viability of the seeds was assessed via cut tests, using a single-sided razor blade to bisect the seeds longitudinally (along the axis of the cotyledons). Seeds with plump endosperm and an embryo were assumed to be viable.

In addition to population census field work, visits were made to local historical museums in the Butte Creek Canyon (Centerville Recreation and Historical Society) and Three Rivers area (Three Rivers Historical Society) to obtain information on early European settlers of these regions, and thereby ascertain whether *D. virginiana* was deliberately planted by these settlers.

RESULTS

A total of six sites were visited in four counties (Table 1, Appendix 1). Live *D. virginiana* plants were found at each site, although the geographic extent of the populations, the size distribution of the plants, and their success in producing viable seeds was highly variable (Table 1). At the Lawson Creek site in San Diego County (D. Burge 2217, Table 1), only one individual was observed, and this plant was most likely the survivor of an orchard at an abandoned home. The plant did not show evidence of clonal or sexual reproduction. At the Mill Creek Canyon site (D. Burge 2210; Table 1), few plants were found, and there was evidence that the site was once the location of a homestead or other type of settlement, suggesting that the trees might have been planted. In addition, this site was apparently affected by a recent fire, which killed many of the mature trees, as evidenced by the presence of downed, partially burned trunks. No evidence of seedling recruitment was noted, though viable seeds were recovered from mature fruits on one tree (Table 1). At the second site in San Diego county (D. Burge 2215; Table 1), in the city of Alpine, the population consisted of 10 mature individuals growing in a roadside ditch, several producing viable seeds. However, no seedlings or saplings were observed at this highly modified site, which appears to be actively managed via mowing and vegetation removal. Similar to the San Diego County site at Alpine, the second San Bernardino County site (D. Burge 2211; Table 1) consisted of a few individuals, though of a more diverse age class than at the Alpine site, suggesting some form of reproduction. At the remaining sites in Butte County (D. Burge 2184; Fig. 1) and Tulare County (D. Burge 2202; Fig. 1), relatively extensive populations of variable sized trees were found, with very little evidence of human influence. Nearly all of the most mature trees in these two populations produced fruits with viable seeds (Table 1). At the site in Tulare County, the population formed a virtual monoculture, *D. virginiana* being almost the only plant species, with dozens of saplings per square meter (Burge personal observation).

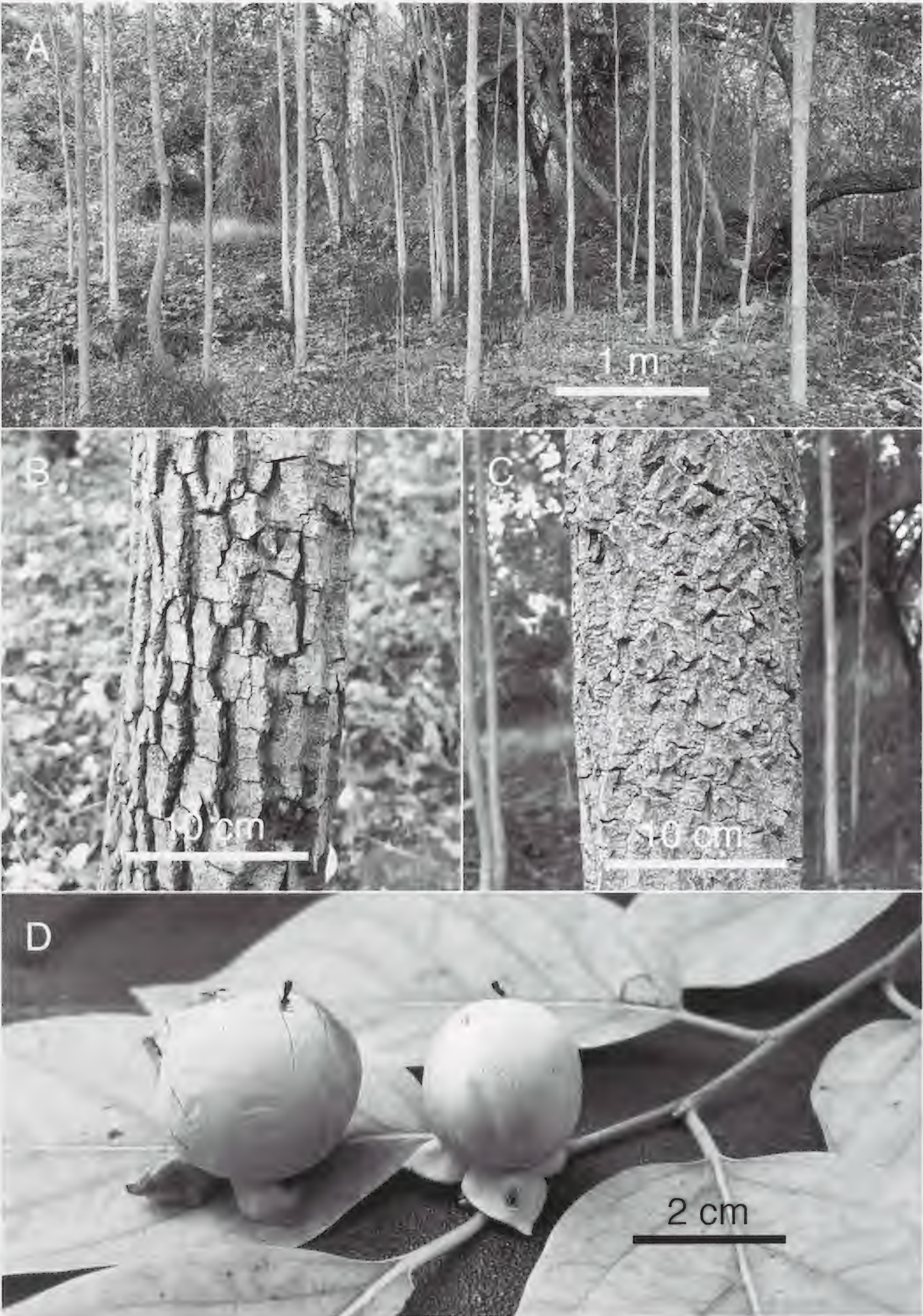


FIG. 1. Representative populations of *D. virginiana* observed in 2016. A–C, South Fork Kaweah River, Tulare County, CA (D. Burge 2202); D, Centerville, Butte County, CA (D. Burge 2184).

DISCUSSION

Diospyros virginiana does not grow indigenously west of Texas, and was therefore most likely introduced to California by humans, probably for use as food (Hrusa et al. 2002). Non-indigenous people of generally European ancestry began to arrive in California in large numbers in the mid-nineteenth century, bringing with them many hundreds of non-native organisms. The descendants of these introductions have become the naturalized flora of California, which is still actively growing, both in terms of diversity and occupied space (Baldwin et al. 2012). *Diospyros virginiana* found outside of cultivation in California is probably descended from plants brought by settlers, possibly even cultivars that are still occasionally grown in the eastern United States (Briand 2005). This idea is to some extent corroborated by the association of *D. virginiana* populations in California with known settlements dating back to the mid-nineteenth century. For example, the Centerville population in Butte County (D. Burge 2184; Fig. 1) occurs at the same location as the now defunct Gold Rush era village of Centerville (Colman 1972). The Kaweah River population in Tulare County (D. Burge 2202; Fig. 1) is also associated with mid to late nineteenth century settlements (Britten 2013; McKee 2013). In fact, recent historical research carried out in the Three Rivers area (McKee 2013; McKee et al. 2017) has revealed that one early settler on the South Fork of the Kaweah River, Ira J. Blossom (1832–1924) was known to plant a variety of fruit trees, which were obtained from USDA Plant Introduction Stations. However, the exact trees species he planted and the stations where these trees originated are not known.

The work presented here provides support for the idea, first proposed by Hrusa et al. (2002), that *D. virginiana* is a naturalized component of the California flora. The wide-ranging size distribution observed in the Butte and Tulare County populations (Table 1), as well as the presence of many apparent seedlings and saplings in both locations, suggests that the populations are self-sustaining. Furthermore, production of viable seeds by trees at all but one of the visited populations suggests that sexual reproduction is taking place. Wild *D. virginiana*, like all other members of the genus *Diospyros*, is dioecious, and females are not known to form seeds unless pollinated. It is therefore evident either that 1) both male and female trees are present in these populations, or 2) that these populations are descended from a perfect-flowered form, examples of which have been developed by tree breeders (Briand 2005). It would be desirable to visit the plant populations in the spring to determine the sex of the trees, and thereby verify if these plants are perfect flowered, as such a condition, if stable, would make these plants much more likely to spread than if they are dioecious (especially if they were capable of self-pollination). In the latter case, both male and female

plants would need to become established in a new location for the population to have a chance of sexual reproduction.

Diospyros virginiana does not have many stations outside its native range, and has never been documented as weedy or invasive (*sensu* Richardson et al. 2000). However, its native range it is an early successional invader of abandoned pastures and other disturbed areas (Briand 2005), and has a deserved reputation as a nuisance species due to its propensity to resprout from the crown or roots, making it difficult to eradicate from areas where it is not desired (Briand 2005). Although this species does not appear to be invasive in the ecological context where it was observed in the course of this study, census data suggests that the Butte County and Tulare County populations are self-sustaining and likely spreading. Known animal dispersers of this species are present in California (Briand 2005; Boone et al. 2015), and could facilitate the spread of this plant. Given the association of this species with water courses, additional populations will probably be discovered in riparian areas along the banks of rivers and streams, probably in the vicinity of current or former human habitation. In addition to elucidating the mating system of the naturalized populations, future research should establish where else this plant has become naturalized and determine what effect its presence has on the natural ecosystem in the locations where it is presently found.

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B. Baldwin and E. Dean provided helpful discussion of the status of *D. virginiana* in California. Several anonymous reviewers provided helpful comments on drafts of this work. V. Church provided logistical support for this research. Thanks are also due to the Three Rivers Historical Society and the Centerville Recreation and Historical Society for assistance with historical aspects of this research.

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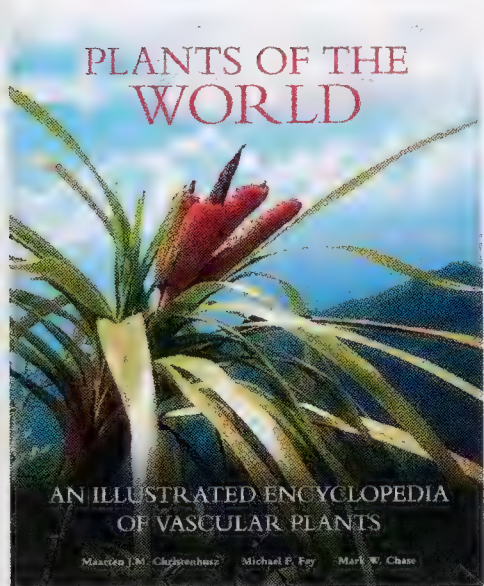
APPENDIX

SAMPLED *DIOSPYROS* POPULATIONS

For each sampled population (Table 1), the format is as follows: collector name and number (herbarium of voucher specimen deposition), description of locality, county, GPS coordinates, County, State.

DIOSPYROS VIRGINIANA—D. O. Burge 2184 (DAV), 22 Oct 2016, Centerville area, Butte Creek watershed (GPS [NAD84]: 39.79658, –121.6485), Butte Co., CA. D. O. Burge 2202 (DAV), 20 Nov 2016, South Fork Kaweah River, roadside on South Fork Drive (GPS [NAD84]: 36.40577, –118.88623), Tulare Co., CA. D. O. Burge 2210 (DAV), 10 Dec 2016, Mill Creek Canyon, near Mountain Home Village (GPS [NAD84]: 34.09738, –116.97832), San Bernardino Co., CA. D. O. Burge 2211 (DAV), 10 Dec 2016, San Bernardino Mountains, Old Mormon Road Monument (GPS [NAD84]: 34.23316, –117.29418), San Bernardino Co., CA. D. O. Burge 2215 (DAV), 17 Dec 2016, Alpine, roadside on Alpine Boulevard (GPS [NAD84]: 32.83438, –116.75354), San Diego Co., CA. D. O. Burge 2217 (DAV), 17 Dec 2016, Lawson Creek, S of confluence with Sweetwater Creek (GPS [NAD84]: 32.76468, –116.79545), San Diego Co., CA.

Review



Plants of the World. An Illustrated Encyclopedia of Vascular Plants, By M. J. M. CHRISTENHUSZ, M. F. FAY, AND M. W. CHASE. 2017. Kew Publishing, Royal Botanic Gardens, Kew, United Kingdom and The University of Chicago Press, Chicago, IL. 816 pp. ISBN 978-1-84246-6346 (hardcover). Price \$95.00, hardcover illustrated edition; \$76, Kindle edition.

Literature resources that cover descriptions of all plant families have been few and very far between over time—Engler and Prantl's, *Die Natürlichen Pflanzenfamilien*, was published from 1887 to 1915; Hutchinson's *The Families of Flowering Plants* in its 3rd edition was published in 1973; Cronquist's *An Integrated System of Classification of Flowering Plants* was published in 1981. While these treatments remain of historical interest, plant classification—especially that of the angiosperms—has changed mightily over the recent decades, and continues to do so. This is largely due to the advent of DNA-based phylogenetic data that have become commonplace and affordable. The work of the Angiosperm Phylogeny Group (APG) website, which has utilized these data, has been the principal resource for reviewing phylogenetic relationships of plant families since the early 2000s. Now, a new print resource exists that covers the families of vascular plants—every one of them—conforming *almost* entirely to the treatment produced by the APG IV. It is likely to become a major reference for botanists well into the future.

The meat of the book is an evolutionary sequential treatment, order by order, of all 451 families from Lycopodiales to Apiales. Each one is illustrated with one or more high quality photos. Very few treatments rely solely on a line drawing or herbarium specimen. Although the details are dense, even the lay reader is likely to find the writing very accessible *and* the scientific information has not been compromised. For those of us who are obsessed with family-level taxonomy, the book is pure fun.

The first item is a two-page layout of a sample treatment titled “How to use this book.” Then before getting to the family treatments, there are brief introductory chapters on Evolution of Land Plants, Plants and Human Culture, Naming Plants, Classification and the APG, Fossil Plants, Families, Genera, Phytogeography, and Economic Botany.

Of particular interest, for the lay reader and scientist alike, is the informal ‘Tree of Life’ presented on p. 11. This is a cartoon drawing depiction of what appears to be a deciduous tree, where each branch accurately indicates the relationships of all terrestrial green plant orders. The main trunk is the central phylogenetic backbone. Major groupings above order (i.e., lycopods, ferns, gymnosperms, and angiosperms) are artfully depicted by green leaves surrounding the appropriate orders. There are even numbers at the “branching” nodes indicating in millions of years, when each order emerged in the distant past.

Following the introductory chapters, each family is grouped into its appropriate order and then detailed with sections on: key characteristics, sometimes with exceptional or errant characters included; distributional range, which indicates the family's natural range on a worldwide map; phylogeny and evolution, which includes discussions of previous systematic placement(s) of the family; an alphabetical list of genera with the number of species within each genus; uses by humans, which is often extremely extensive (e.g., the entry for Asteraceae goes on for six pages, and if you have questions on *Citrus* species and cultivars, find the answers here); and finally there is a brief etymology of the family name. Each family is given a common name, and while it remains uncertain how useful these will be, common family names should attract the attention of readers who want to know more about the jumbie-pepper family, the snake-mushroom family, the wantsum family, and so on.

In addition, scattered across the book are discussions of interest peculiar to certain families where the authors warn that “it is important to know your taxonomy!”. Samples include “rosehip cruelty” (Rosaceae); “from the seed of a hanged man” (Solanaceae); “I see you — man-eaters?” (Fouquieriaceae); “crying flowers” (Actinidiaceae); “mistaking *Veratrum* for *Gentiana*, with fatal consequences.” Other occasional entries are the vignettes highlighting famous taxonomists who made major contributions to understanding plant family relationships. Among these, a brief bio-sketch of Engler and Prantl appears in the treatment for Loranthaceae (*Englerina* is a genus in that family). Another biosketch of Art Cronquist, who passed away while examining herbarium specimens of *Mentzelia*, is placed with the Loasaceae.

Plant family records are included where appropriate. For example, *Polylepis tarapacana*—highest elevation for a tree (5100 m); *Lomatia tasmanica*—oldest clone (43,600 years); *Ranunculus lobatus*, *Arenaria bryophylla*, and *Solms-laubachia himalayen-*

sis—highest elevation (7,756 m); *Silene stenophylla*—longest seed dormancy (31,800 years).

Past the family treatments there is a 32-page glossary nicely illustrated with high quality photographs; although the scope is impressive, the authors reference the even more complete Kew plant glossary (Beentje 2010). While most academics will not need a glossary, it will be very welcome by the general reader. The book is completed with a Further Reading section, a family-by-family bibliography to get you closer to each taxon. There is also a nice set of general references.

There are likely to be places in the book where what is written may not be consistent with the reader's understanding of the family. For example, Polemoniaceae are described to have unequal stamens. This is true for *Phlox* and *Microsteris*, but most genera in the family have equal stamens. Further, botanists used to the Jepson Manual will read that the family contains only 18 genera, and may be puzzled to note that *Leptosiphon*, *Microsteris*, *Loeseliastrum* (etc.) are not listed. This is curious, as the publication responsible for the delineation of genera in the family (Porter and Johnson 2000) is cited in the Further Reading, but it isn't followed in the list of genera in the family. Whether this is an oversight or simply a preference by the authors to follow an older taxonomy is unclear.

In the case where the book does not agree with APG IV, there is only a small divergence concerning only one genus (*Peltanthera*) and the APG IV recognized family of Calceolariaceae. The authors argue and place these two taxa in an expanded Gesneriaceae. Their rationale for this is based on distinguishing morphological characters and is explained in the introduction and in great depth under the description of their expanded Gesneriaceae.

Sadly, the liverworts, hornworts, and moss orders are not treated in this book. They are depicted in the formal phylogeny (p. 10), informally in the 'Tree of Life' near the base of the tree trunk (p. 11), and mentioned in the brief Evolution of Plants chapter

(p. 3), wherein a representative photo of each appears along the bottom of the page. It's curious that such a detail-oriented book that spans the scope of nearly all terrestrial land plants would choose to leave out the earliest diverging orders from their comprehensive—and frankly, quite impressive—treatment of all families of terrestrial plants.

Regardless, with or without the non-vascular plant orders, this is a big book. *Plants of the World* measures 9.5×11.5 inches, is 2 inches thick, and weighs in at 3.226 kg. For those of you that like U.S. customary units, that's a hefty 7.112 pounds! The production is of excellent quality, with an abundance of photos. The authors state the book is intended for a general audience; we believe it is a goal accomplished. It has not one, but two bookmark ribbons (one green, one yellow) that are necessary to hold onto multiple ventures deep into the many rabbit holes of the great diversity in the book *Plants of the World*.

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MADROÑO

A WEST AMERICAN JOURNAL OF BOTANY

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NOTEWORTHY COLLECTION

CALIFORNIA

SANICULA SAXATILIS E. Greene (APIACEAE).— Santa Clara Co., Santa Cruz Mts., Mt. Umunhum (Midpeninsula Open Space District Sierra Azul Preserve), east flank of its NE Ridge, 37.15958, -121.89200, 730 m, *J. Rawlings 1347*, 10 April 2015 (JEPS100530); summit area *J. Rawlings 1493*, 29 April 2015 (SJSU15802); east face *J. Rawlings 1721*, 12 April 2016 (SJSU16001). 100s of plants, elev. 730 - 1036 m, on rocky outcrops and well-developed scree gullies. Full extent of population is not yet known; portions of the NE ridge with potentially suitable habitat were not surveyed because of steep, friable, rock faces. Associated species include *Allium falcifolium* Hook. & Arn., *Claytonia exigua* subsp. *exigua* Torr. & A. Gray, *Lomatium macrocarpum* (Torr. & A. Gray) J. M. Coult. & Rose, *Leptosiphon ambiguus* (Rattan) J. M. Porter & L. A. Johnson, *Minuartia douglasii* (Fenzl ex Torr. & A. Gray) Mattf., and *Selaginella bigelovii* Underw.

Previous knowledge. Rock sanicle had been known only from the Inner Coast Ranges in the summit rock community around peaks and high ridges of Mount Diablo and Mount Hamilton (Diablo Range, Contra Costa Co., and Hamilton Range, Santa Clara Co.) (Jepson Flora Project eds. 2018).

Significance. The species has a California rare plant rank of 1B.2 and is listed by the State of California as rare. John H. Thomas (1961) called out the Inner Coast Range affinities of a number of taxa growing on the eastern slope of Sierra Azul in the Santa Cruz Mountains (including Loma Prieta and Mt. Umunhum summits). These taxa include *Allium falcifolium*, *Arctostaphylos glauca* Lindl., *Boechera breweri* (S. Watson) Al-Shehbaz, *Chaenactis glabriuscula* DC., *Chorizanthe membranacea* Benth., *Clarkia breweri* (A. Gray) Greene, *Ericameria nauseosa* (Pall. ex Pursh) G. L. Nesom & G. I. Baird var. *speciosa* (Nutt.) G. L. Nesom & G. I. Baird, *Erigeron petrophilus* Greene, *Eriogonum saxatile* S. Watson, *Garrya fremontii* Torr., *Hulsea heterochroma* A. Gray, *Lewisia rediviva* Pursch, *Mentzelia lindleyi* Torr. & A. Gray, *Myriopteris intertexta* (Maxon) Grusz & Windham, *Pinus sabiniana* D. Don, *Rhamnus ilicifolia* Kellogg, *Selaginella bigelovii*, *Viola purpurea* Kellogg subsp. *quercetorum* (M. S. Baker & J. C. Clausen) R. J. Little; all have been found on Mt. Umunhum. *Hulsea heterochroma* was vouchered in 1952 (CAS-BOT-BC311952), but not found during our survey. All other species listed were documented by the authors. The main peak of Mt. Umunhum (near the

abandoned military radar facility – a large cement block-shaped structure) “consists of a complex mix of rocks, mostly serpentinite, associated with the Coast Range Ophiolite” (Stoffer 2005). This “disrupted assemblage of rocks which originally formed the Mesozoic convergent continental margin” (McLaughlin 2002) is frequently puzzling, especially in zones of extensive rock slides, to botanists interested in the substrate of their plants. The upper approximately 305 m of elevation of the 1,063 m mountain was surveyed during 2015–2017 and 400 herbarium sheets were made, most deposited in the Sharsmith Herbarium, San Jose State University, San Jose, CA. The results demonstrate the vascular plant richness of the approx. 200 hectare study area and sky island in the Santa Cruz Mountains, and further support Stebbins’ and Major’s (1965) identification of the region from Año Nuevo Point to Loma Prieta Peak as a “botanical hotspot” of endemism and speciation in the Bay Area and California: 362 Taxa, 71 Families, 8 CNPS Ranked, 80% Native (Hickman and Rawlings 2018; Hickman 2018).

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NOTEWORTHY COLLECTION

CALIFORNIA

CAMPANULA ANGUSTIFLORA Eastw. (CAMPANULACEAE).—El Dorado Co., Volcanoville, 38.971111, -120.821111, 792 m, El Dorado National Forest Road 13N56, between Cock Robin Point (west), Jakeys Hill (east), Otter Creek (south), and American River Middle Fork (north), found near the perimeter of the Trailhead Fire (June 2016) scar, along ridgeline, growing among burned *Quercus*, *Pinus*, *Arctostaphylos nissenana* Merriam, and *A. viscida* Parry, some isolated individuals, many emerging in cache-like clusters, 100+ plants observed, 8 May 2017, Steven Serkanic 173 (DAV 223968).—Mendocino County, Anchor Bay, 5.8 km east of Hwy 1 on Iversen Road, on the first ridge east of the coast, 38.851411, -123.594334, 350m, on compact sand under shrubs, with *Arctostaphylos nummularia* A.Gray, *Pseudotsuga menziesii* (Mirb.) Franco, *Quercus wislizeni* A.DC., *Pickeringia montana* Nutt., \pm 25 plants seen, May 5, 2015, Nancy Morin 701 (UC2070664), annual, 5–20 cm tall, leaves oblong-ovate, 4.5–9 mm, leathery, flowers pedicelled, sepals erect, converging in fruit, corolla 2.5–6 mm, cylindrical, pale blue to white, the lobes suberect, capsule spheric, strongly ribbed, with pores near the middle of the capsule.

Previous knowledge. *Campanula angustiflora* is known from only about 20 localities, all in northern and central California (Morin 1980). It has been reported from the inner North Coast Range and the eastern edge of the Outer North Coast Range from Lake and Napa counties, easternmost Sonoma County, and westernmost Solano and Colusa counties; outlying populations are in the San Francisco Bay Region in southernmost Marin County on Mount Tamalpais and northern Santa Cruz County (Morin 2012). *Campanula angustiflora* occurs in

chaparral, on serpentine soils, and in recently burned areas.

Significance. The El Dorado Co. collection is the first locality reported in the Sierra Nevada, 130 km east of the next closest population, which is in western Colusa County; it is in an area that burned in June of 2016. The Mendocino County collection is the first report from the North Coast Subregion (Baldwin et al. 2012), 63 km west of the next closest population, which is in Lake County. It occurs in vegetation similar to the *Hesperocyparis pigmaea* (Lemmon) Bartel alliance (CNPS 2018) found further north near Albion and Fort Bragg, on compact, white sand.

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POST-FIRE ECOPHYSIOLOGY OF ENDEMIC CHAPARRAL SHRUB SEEDLINGS FROM SANTA CATALINA ISLAND, SOUTHERN CALIFORNIA

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ABSTRACT

Island systems are generally more sensitive to the introduction of non-native species than comparable mainland systems and are particularly vulnerable to disturbances that create opportunities for invasion. We examined the impact of browsing by introduced deer on seedling establishment and physiology in three obligate seeding (i.e., non-resprouting) species on Santa Catalina Island for two years following a large fire in 2007: *Arctostaphylos catalinae* P. Wells (Ericaceae), *Ceanothus arboreus* E. Greene (Rhamnaceae), and *Ceanothus megacarpus* Nutt. var. *megacarpus* (Rhamnaceae). Four post-fire sites were established, with each site containing 12–20 plots exposed to browsing and 12–20 control plots protected from deer browsing. Browsing did not significantly affect seedling survival or physiology in *C. arboreus*, the only species to experience significant browsing; browsing did, however, significantly reduce seedling height. Predawn and midday water potentials, stomatal conductance, and growth differed among species during their first post-fire dry season. *Arctostaphylos catalinae* experienced the lowest dry season predawn water potentials (–3.3 MPa compared to –1.7 MPa for *C. arboreus* and –2.2 MPa for *C. megacarpus*). *Ceanothus arboreus* and *C. megacarpus* exhibited relatively high seedling survival (56% and 69%, respectively) and high resistance to xylem cavitation of stems (P_{50} = –6.0 and –6.7 MPa, respectively), compared to *A. catalinae* which had lower seedling survival (30%) and was less resistant to cavitation (P_{50} = –4.2 MPa). Browsing significantly impacted seedling height, which could impact stand structure. Differential browsing pressure and seedling resistance to water limitation may have important consequences for long-term distribution and abundance of these island endemics on Santa Catalina Island.

Key Words: Drought, introduced species, life history type, mediterranean-type ecosystem, mediterranean-type climate region, vulnerability to cavitation.

Crown fires are a recurrent disturbance in the chaparral shrublands of southern California. Following a fire, chaparral shrubs persist through vigorous resprouting and fire-stimulated seed germination and seedling establishment. Woody shrub species are typically classified as falling into one of four different life history types in response to fire: obligate seeders, which persist only through the germination of fire-cued seeds; facultative seeders, which persist through a combination of resprouting from underground structures, as well as germination of fire-cued seeds; obligate resprouters, whose seeds are killed by fire and persist through resprouting; and opportunistic species, the adults and seeds of which are killed by fire but readily disperse seeds into burned sites from adjacent unburned areas. Under many conditions, mediterranean-type shrublands rapidly recover following fire and maintain their pre-fire composition (Hanes 1971).

Post-fire regeneration of chaparral stands may fail when the fire regime changes or if additional disturbances occur in tandem with fire. For instance, a change in the fire regime that reduces the time interval between successive fires (i.e., short fire return

intervals) may alter chaparral shrub community compositions or convert shrublands to alien grass-dominated savannahs or sage scrub communities (Zedler et al. 1983; Haidinger and Keeley 1993; Jacobsen et al. 2004; Lippitt et al. 2013). The occurrence of additional disturbances, including the addition of browsing by mammals (Howe 1981; Mills 1983; Quinn 1986; Moreno and Oechel 1991; Tyler 1995; Ramirez et al. 2012), drought (Pratt et al. 2014), or pathogens (Jacobsen et al. 2012), during the post-fire recovery period may also impact shrub community composition.

Many of these interactions and their subsequent impacts on chaparral shrub communities may be greater for island chaparral systems compared to mainland systems, particularly in the case of fire and herbivore interactions. This may be due to an increase in the vulnerability of island plants to being browsed (Bowen and van Vuren 1997; Vourc'h et al. 2001), to the population dynamics and behavior of introduced mammalian herbivores on islands that do not have natural predators (Knapp 2005; Stapp et al. 2006; Laundré et al. 2010), or to intense herbivory of post-fire areas, particularly when fires are small

TABLE 1. THE GPS LOCATIONS, ELEVATION, AND NUMBER OF PLOTS FOR FOUR SITES THAT WERE ESTABLISHED IN AN AREA THAT BURNED IN MAY 2007 ON SANTA CATALINA ISLAND, SOUTHERN CALIFORNIA. Within each site, multiple $1 \times 1 \text{ m}^2$ plots were established in areas that were protected from large mammal browsing by tall fences (Fenced plots) or were established in areas not protected by fences and that were open to being browsed (Browsed plots).

Site	Dominant obligate seeding shrub species	GPS location (°)	Elevation (m)	Browsed plots (#)	Fenced plots (#)
1	<i>Arctostaphylos catalinae</i>	33.3539, -118.3584	420	20	20
2	<i>Ceanothus arboreus</i>	33.3568, -118.3471	315	15	12
3	<i>Ceanothus arboreus</i>	33.3501, -118.3430	280	12	12
4	<i>Ceanothus megacarpus</i> var. <i>megacarpus</i>	33.3491, -118.3656	430	20	20

(Quinn 1986; Knapp 2005). Additionally, for the location of the present study, the fire history on Santa Catalina Island is unknown and the fire return interval is assumed to be longer than the mainland, which may affect the ability of the vegetation to recover. The fire-regime in mainland southern California is a late summer-fall regime with fire return intervals of 30-100 years (Keeley et al. 1999; Keeley and Fotheringham 2001), but the fire regime on the island may be characterized by longer intervals between fires due to the rarity of lightning ignitions (Carroll et al. 1993).

Fire and browse may impact both the recovery of resprouting species and the recruitment of seedlings. For Santa Catalina Island in southern California, the negative impact of fire combined with large mammal browsing on post-fire recovery of chaparral community structure has already been demonstrated for resprouting species. A recent study of post-fire chaparral species with resprouting ability found that browsing by introduced mule deer (*Odocoileus hemionus* Rafinesque) lead to high mortality of resprouting shrubs (Ramirez et al. 2012). Browse-related reductions in plant crowns for three dominant species of resprouting shrubs at this site resulted in a more than 93% reduction in the canopy coverage (Ramirez et al. 2012). The period of this study also coincided with an extremely dry year, with only 130 mm of rain falling during the 2006-2007 rain-year compared to an 80-year average of 323 mm (Ramirez et al. 2012). Post-fire browsing by mammalian herbivores has also been shown to impact the post-fire vegetation recovery patterns of seedlings in mainland chaparral communities (Mills 1983; Mills 1986; Quinn 1986; Frazer and Davis 1988; Thomas and Davis 1989; Tyler 1995), but has rarely been studied on southern California islands, in part because of infrequent fires (Carroll et al. 1993; Duncan and King 2009). Following a small fire on Santa Catalina Island in 1999, two seeding species were reported to be sensitive to browse, although detailed data were not reported (Knapp 2005).

A large fire, which occurred in May 2007 and burned about 10% of the land area on Santa Catalina Island, provided us with the opportunity to examine how browse by introduced large mammalian herbivores may impact post-fire recovery of obligate seeding species. We were interested in examining whether obligate seeding species of chaparral shrubs in island ecosystems would success-

fully recover following this fire, particularly in the context of potential co-disturbance with browsing from introduced mule deer. Having already observed the severe effect browsing could have on the survival of large obligate resprouting species (Ramirez et al. 2012), we hypothesized that seedlings, being small and more delicate than an established resprout, would be more vulnerable to deer browsing and associated disturbance (trampling). We examined post-fire recovery of three shrub species by following seedling survivorship and several physiological and hydraulic parameters in plots not protected from deer browse and sites that were protected from deer, the only large mammalian browser currently present on the island.

METHODS

Santa Catalina Island lies 22 miles (35 km) off the coast of southern California and contains several vegetation types, including stands of maritime chaparral (Westman 1983). A large fire occurred in May 2007, which burned about 10% of the land area on Santa Catalina Island. Within the burned area, we established 4 sites (Table 1), each of which was dominated by one of four island endemic chaparral shrub species or subspecies: *Arctostaphylos catalinae* P. Wells (Ericaceae) (site 1), *Ceanothus arboreus* E. Greene (Rhamnaceae) (sites 2 and 3), and *Ceanothus megacarpus* Nutt. var. *megacarpus* (Rhamnaceae) (site 4). All of these species are evergreens that are obligate seeding and non-resprouting after fire. Sites were selected based on the presence of these species as identified in adjacent unburned areas and from burnt stumps within the burned areas. Sites were surveyed to estimate the pre-fire density of these species within each site by surveying and recording the burnt stumps and root crowns within each site. Although these sites were largely dominated by our focal obligate seeding species, there were also resprouting evergreen shrub species present in low densities, including *Adenostoma fasciculatum* Hook. & Arn. (Rosaceae), *Crossosoma californicum* Nutt., *Heteromeles arbutifolia* (Lindl.) M. Roem. (Rosaceae), *Malosma laurina* (Nutt.) Abrams (Anacardiaceae), *Quercus pacifica* Nixon & C.H. Mull. (Fagaceae), *Rhamnus pirifolia* Greene (Rhamnaceae), and *Rhus integrifolia* (Nutt.) Rothr. (Anacardiaceae).

At each site we established 12-20, $1 \times 1 \text{ m}$ randomly distributed permanent plots in an approx-

imately 500 m² area exposed to browsing and 12-20 randomly distributed permanent plots in an approximately 500 m² area protected from browsing by a tall plastic deer-proof fence with metal reinforcement along the lower few feet. The fences restricted access by large animals (deer and bison are both present on the island and in the study areas), whereas small terrestrial animals and birds were not restricted. Since bison are grazers and do not regularly browse woody vegetation (although we have observed some browsing by bison), these fences were primarily to limit deer access to shrubs, but also limited trampling by deer and bison.

Fenced plots were contained within or near larger fenced areas within the burned areas that were installed shortly after the fire by the Catalina Island Conservancy to protect sensitive and rare species from the intense deer browse that had previously been observed within a smaller burned area (Ramirez et al. 2012). For our sites, the two *C. arboreus* sites (sites 2 and 3) and the *A. catalinae* site (site 1) were located within larger fenced plots that were established by the Catalina Island Conservancy. To decrease the likelihood of fence breaches, in two of these sites, particularly where bison activity was high, we isolated our study areas using additional fencing to completely enclose only our study area within its own layer of fencing. We selected our sample areas to occur at fenced area margins where we could also sample an immediately adjacent unfenced area of similar aspect and pre-fire species composition. For *C. megacarpus*, we installed a fence specifically for the present study and this fenced area was not part of a larger fenced plot. All sites were located fairly close to one another, with the two most distant sites being 2.7 km apart and the other sites occurring in between.

Sites were established in February-March 2008 and plots were monitored through fall 2009. When plots were established, some seedlings had already germinated, but others were still germinating following the post-fire winter wet period. Monitoring occurred approximately monthly the first year and every three months during the second year of sampling. The 2007-2008 rainfall year, during which seeds would have been germinating, was similar to average (332 mm compared to an 80-year average of 323 mm from a nearby weather station; Ramirez et al. 2012). The second year of the study, 2008-2009, was drier than the first year, with only 196 mm of rainfall. Rainfall totals were obtained from a weather station located within 200 m of site 1 (www.wrcc.dri.edu).

At each sampling visit, growth and survival were monitored. Each 1 × 1 m plot was divided into 10 cm × 10 cm cells using grids and all of the surviving seedlings in each cell were recorded for every plot during each sampling date. We subdivided the 1 m by 1 m plots into these smaller grids so that we could more easily track individual seedlings. We also recorded whether each seedling was browsed or

not. The heights of 10 randomly selected seedlings were measured within each plot at each visit. A different set of seedlings were randomly selected for these measures at each sampling time.

During each sampling period, a series of physiological measurements were also conducted. Some of measurements involved the destructive harvesting of plant material (e.g., water potentials) and they were therefore conducted on plants that were not within our permanent plots. Instead, plants occurring within fenced and non-fenced areas adjacent our plots were randomly selected, with different seedlings selected for each measurement period. Only one site was included for each species, with measurements conducted at sites 1, 3, and 4. During each sampling date, both predawn and midday water potentials were measured on 6-12 individuals each from within fenced and non-fenced areas of sites (Pressure chamber, Model 2000, PMS Instruments, Albany, OR, USA). Dark-adapted chlorophyll fluorescence (F_v/F_m) was also measured in the morning, with cuvettes attached to one leaf each of 6-12 individuals from within fenced and non-fenced areas for each species. This parameter measures the intrinsic quantum efficiency of PSII and is a sensitive indicator of dehydration stress (Maxwell and Johnson 2000). Values between 0.8 – 0.86 indicate optimum quantum use efficiency and values below 0.8 indicate photoinhibition or reduced quantum efficiency, which is a sign that plants are under strain. Leaves were dark-adapted for 20 min before F_v/F_m was measured using a pulse-modulated fluorometer (OS1-FL, Opti-Sciences, Tyngsboro, MA, USA). Stomatal conductance was measured on 6-12 individuals from within the fenced and non-fenced areas for each species mid- to late-morning (10:00 to 12:30). Fully illuminated leaves were selected for measurements (SC-1 Leaf Porometer, Decagon Devices, Pullman, WA, USA). We found that this device overestimates stomatal conductance by a factor of two when compared to matched leaves using a Li-Cor 6400 (Li-Cor, Lincoln, NE, USA), thus we divided all values by two.

On October 18, 2009, samples were collected for the determination of vulnerability to water stress-induced cavitation from the same three sites used for physiological measurements. Entire seedling shoots were collected at predawn, wrapped in moist paper towels, and placed in plastic bags on ice in a cooler. For all three species, six samples were collected for each species from non-browsed plants near, but not within, our permanent plots. For *C. arboreus*, an additional 6 samples were collected from browsed plants. Browsed samples were not collected from the other two species, because they were not generally browsed and so browsed plants were not available. Samples were kept on ice during transport from the island to California State University, Bakersfield and then refrigerated once in the laboratory. Vulnerability to cavitation curves were then measured over the next three days using the standard centrifuge method

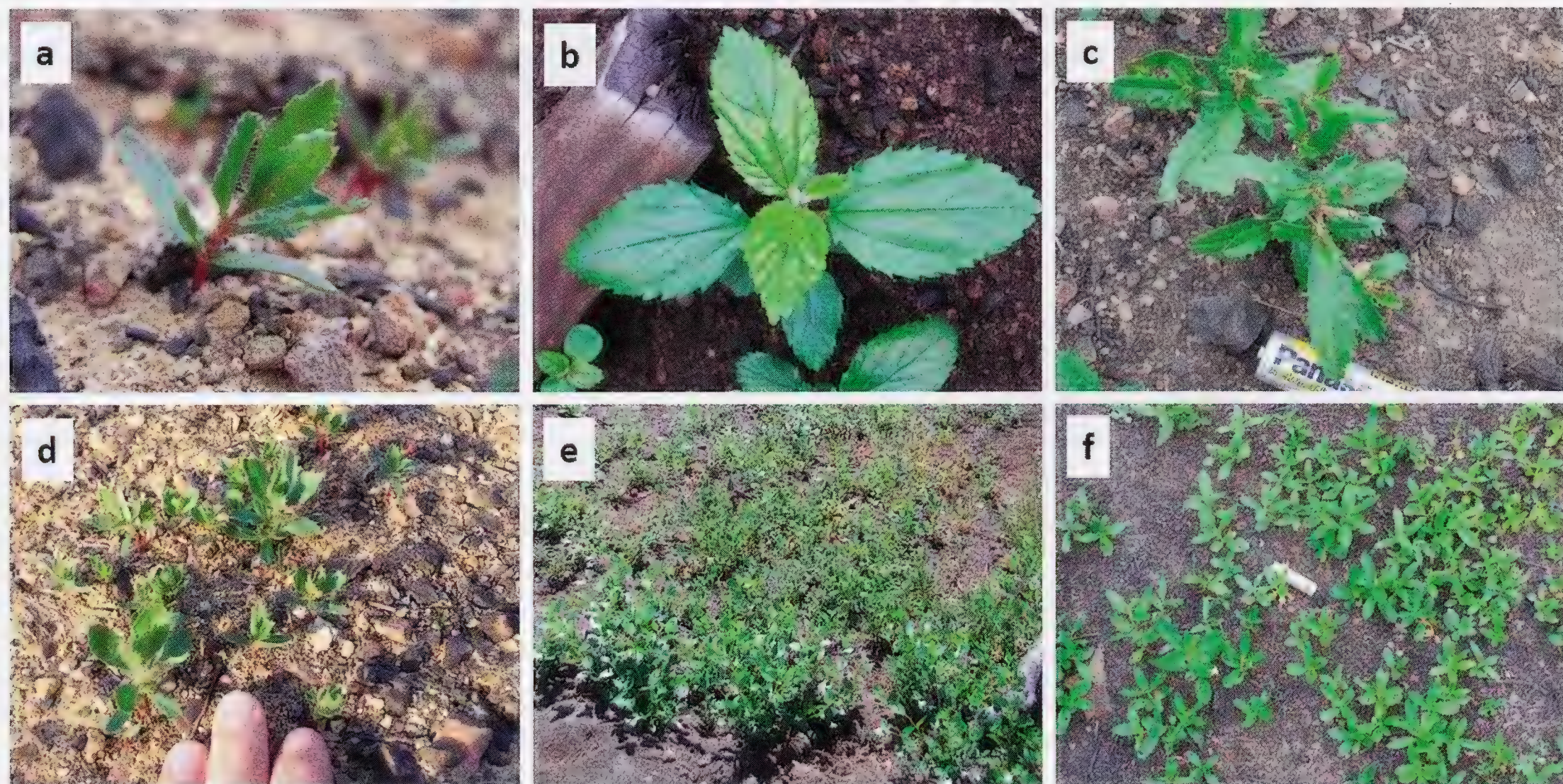


FIG. 1. Post-fire seedlings from three obligate seeding chaparral species on Catalina Island, southern California in April–May 2008: *Arctostaphylos catalinae* (a, d), *Ceanothus arboreus* (b, e), and *C. megacarpus* (c, f). Seedlings were easily identifiable during early seedling emergence (a, b, c) and occurred in near mono-specific stands at relatively high densities (see Table 2 for seedling density measures from the same initial measurement period as shown in panels d, e, f).

(Alder et al. 1997). Samples were flushed for 1 h at 100 kPa with an ultra-filtered (0.1 μm filter) 20 mM KCl degassed solution before being subjected to increasing negative xylem tensions by being spun in a custom centrifuge rotor. Hydraulic conductivity was measured following each spin and these were used to calculate the percentage loss in conductivity at each imposed water potential relative to the initial flushed value. Vulnerability curves were plotted for each sample as the percentage loss in conductivity with declining water potential and were fit using a Weibull curve (Microsoft Excel 2010, Microsoft, Redmond, WA, USA) to calculate the water potential at 50% loss in hydraulic conductivity (P_{50}). Curves were fatigue-corrected (Hacke et al. 2001) using an initial pressure of -0.5 MPa and P_{50} values were calculated using these fatigue corrected curves.

Comparisons of browsed and non-browsed (fenced) plots within a species were only conducted for *C. arboreus*, which was the only species among the three with individuals that were browsed throughout the study. These comparisons were conducted using t-tests when data could be transformed to meet statistical assumptions or using a Mann-Whitney rank sum test if data could not be transformed to meet normality assumptions. Comparisons across species focused specifically on traits measured during the dry season sampling periods during 2008 and 2009. These comparisons were conducted using ANOVAs with Tukey pairwise comparisons, including comparisons of survival among species following the summer dry seasons in 2008 and 2009, predawn and midday dry season water potentials, and P_{50} . Data were tested for

normality and transformed when required or, when transformations were not able to meet the assumption of normality, species were compared using a Kruskal-Wallis ANOVA on ranks with Dunn's pairwise comparisons. All statistics were run using SigmaPlot (11.1.0.102, Systat Software, Inc., Germany).

RESULTS

Plots were established early during and following seedling emergence and seedlings of our target species were recorded in nearly all plots established within our sites (Fig. 1; only six of >120 plots did not have any seedlings of our target species emerge within the plot). Within our established plots, seedlings of our target species were present in high densities, with few other species present (Fig. 1, Table 2). Pre-fire shrub densities were generally very low (Table 2), and even after considerable seedling mortality during the first two dry seasons following seedling emergence (Fig. 2), seedling densities for all three species remained higher than pre-fire adult densities.

Browsing levels varied among species (Fig. 2). We observed only a single deer-browsed *A. catalinae* seedling over the course of this study and very limited browsing of *C. megacarpus* until our final sampling in October 2009, when there was a small increase in browse activity. These low levels of browse were not due to inactivity of deer in the area, because signs of deer activity (prints and scat) were observed across all sites and deer browse (identifiable by browse height and bite patterns) on other co-occurring

TABLE 2. THE PRE-FIRE DENSITY OF DOMINANT SHRUB SPECIES WITHIN EACH SITE AND THE DENSITY OF SEEDLINGS WHEN PLOTS WERE ESTABLISHED DURING THE ONSET OF SEEDLING EMERGENCE (APRIL 2008) AND FOLLOWING THE FIRST (SEPTEMBER 2008) AND SECOND (OCTOBER 2009) DRY SEASONS AFTER SEEDLING EMERGENCE (ACROSS PLOT MEAN \pm 1 SE FROM WITHIN EACH SITE).

Site	Species	Pre-fire adult density (# m ⁻²)	Post-fire seedling density (# m ⁻²)	Post-fire seedling density (# m ⁻²)	Post-fire seedling density (# m ⁻²)
			Apr-08	Sep-08	Oct-09
1	<i>A. catalinae</i>	0.1335	42.9 \pm 8.9	9.2 \pm 2.2	4.6 \pm 1.3
2	<i>C. arboreus</i>	0.0084	50.5 \pm 11.1	26.2 \pm 5.4	21.8 \pm 4.2
3	<i>C. arboreus</i>	0.0081	158.9 \pm 28.8	66.1 \pm 10.0	36.9 \pm 4.5
4	<i>C. megacarpus</i>	0.0055	28.7 \pm 5.2	17.4 \pm 2.9	13.7 \pm 2.4

resprouting species was also observed at all sites. For *C. arboreus*, we observed significant levels of browse in the non-fenced plots, with nearly all unprotected seedlings browsed during some sampling dates. Site 3 experienced two minor fence breaches by deer that resulted in some browse of fenced seedlings, but these breaks were quickly repaired.

Browse drastically reduced the height of unprotected *C. arboreus* seedlings (Fig. 2; final mean height of >60 cm for fenced seedlings and <20 cm for unprotected plants); however, browsing did not affect the survival of seedlings during the period of the present study (Table 3). There was no difference in seedling survival between browsed and non-

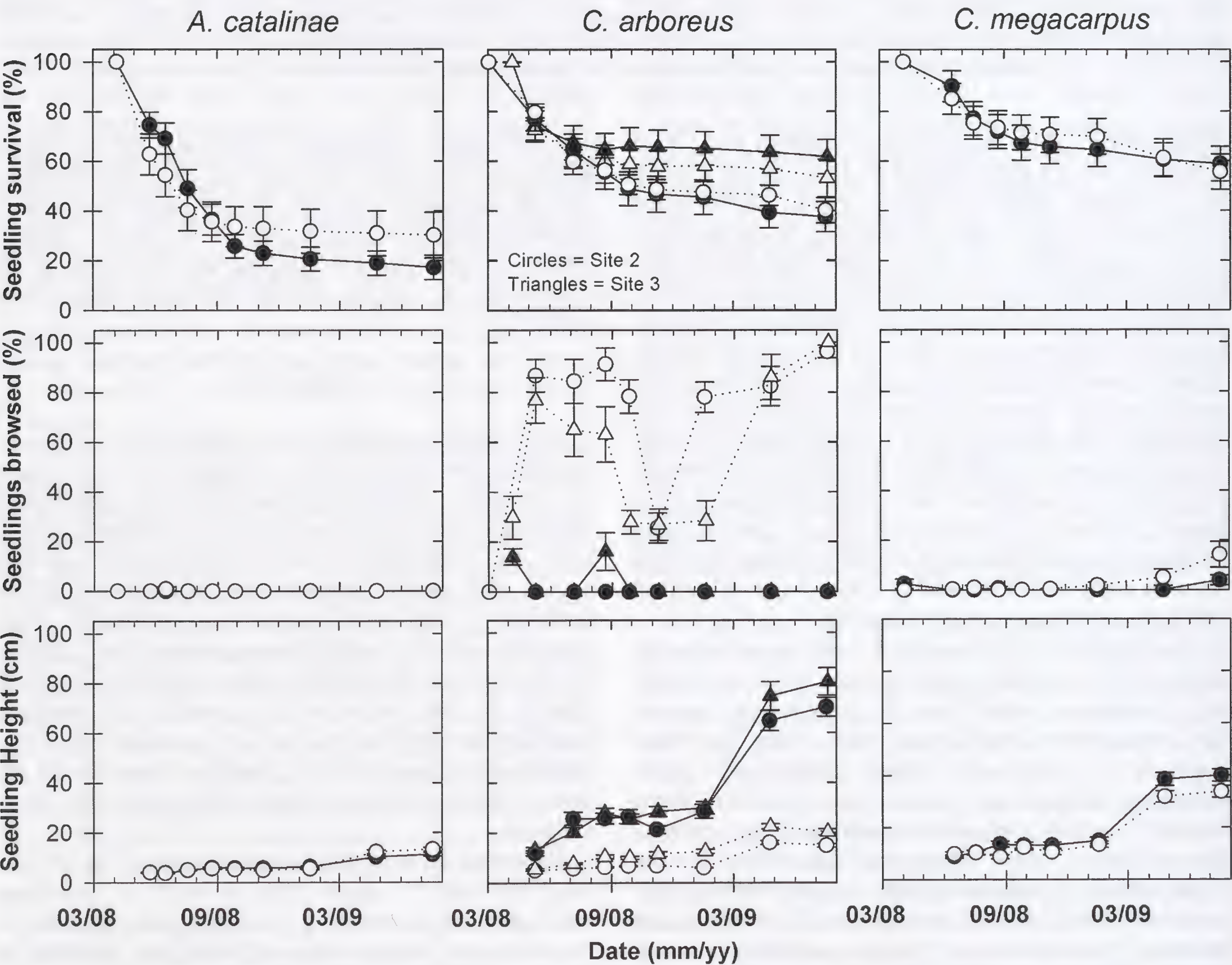


FIG. 2. Seedling survival, the percentage of seedlings browsed by deer, and seedling height of three island endemic chaparral species or subspecies during the first two years post-fire during seedling establishment. Open symbols are from plots that were non-fenced and therefore open to potential browse activity and closed symbols are from plots that were protected from browse by tall fences. Symbols represent means \pm 1 SE. Sites are described in Table 1. There were three minor fence breakages during the course of the study (as seen by the low amount of browse activity for closed symbol data), but these were caught early and quickly repaired, resulting in minimal browse to protected plants.

TABLE 3. DRY SEASON SURVIVAL AND PHYSIOLOGICAL PARAMETERS FOR SEEDLINGS OF THREE SPECIES FROM SANTA CATALINA ISLAND ONE YEAR (2008) AND TWO YEARS (2009) POST-FIRE. Parameters include seedling survival (%), dark-adapted fluorescence (F_v/F_m), predawn and midday water potential (MPa). Maximum seasonal stomatal conductance (g_{smax}) for each year is also shown, with the month during the year corresponding to that maximum value included. Values are means with 1 SE indicated below the mean in parentheses. Different capital letters within a column indicate significant differences between species for the parameter in the column to the left and “ns” indicates that species were not significantly different ($\alpha = 0.05$). An * next to the “fenced” plot parameter for *C. arboreus* indicates that browsed and non-browsed plants were significantly different for that variable and an “ns” indicates that browsed and non-browsed plot did not differ significantly ($\alpha = 0.05$).

Species	Treatment	Survival (%)		F_v/F_m		2008 water potential (MPa)			2009 water potential (MPa)			g_{smax} (mmol m ⁻² s ⁻¹)	
		2008	2009	2008	2009	Predawn	Midday		Predawn	Midday		2008	2009
<i>A. catalinae</i>	non-fenced	33.6 (8.1)	26.7 (8.8)	0.762 (0.014)	0.782 (0.006)	-3.62 (0.11)	-4.14 (0.69)		-2.90 (0.24)	-3.75 (0.18)		June 97.1 (19.5)	January 197.5 (16.6)
	fenced	25.8 (4.8)	11.2 (3.1)	0.721 (0.059)	0.720 (0.028)	-3.04 (0.76)	-3.39 (0.64)		-4.32 (0.63)	-5.43 (0.43)		98.4 (25.1)	185.6 (33.0)
	combined	29.6 (4.5) A	18.8 (4.6) A	0.772 (0.030) A	0.751 (0.016) A	-3.31 (0.62) A	-3.77 (0.46) A		-3.66 (0.39) ns	-4.59 (0.31) ns		97.8 (15.2) A	191.5 (17.7) AB
<i>C. arboreus</i> (site 3 only)	non-fenced	58.8 (6.1)	51.6 (7.6)	0.818 (0.003)	0.777 (0.011)	-1.39 (0.11)	-2.29 (0.14)		-3.89 (0.26)	-3.97 (0.18)		April 411.5 (41.1)	January 190.7 (15.6)
	fenced	66.0 (7.3) ns	60.1 (6.6) ns	0.793 (0.007) *	0.806 (0.003) *	-1.94 (0.16) *	-2.50 (0.16) ns		-4.87 (0.64) *	-5.03 (0.34) *		501.3 (15.4) ns	223.9 (22.8) ns
	combined	62.5 (3.7) B	56.0 (4.6) B	0.811 (0.004) B	0.792 (0.006) B	-1.68 (0.11) B	-2.40 (0.11) B		-4.36 (0.23) ns	-4.50 (0.22) ns		460.4 (27.5) B	207.3 (14.0) B
<i>C. megacarpus</i>	non-fenced	71.5 (7.0)	52.0 (7.0)	0.792 (0.015)	0.749 (0.026)	-2.74 (0.25)	-3.30 (0.74)		-5.13 (0.70)	-5.01 (0.56)		April 63.6 (28.7)	January 147.8 (18.8)
	fenced	67.3 (7.1)	55.7 (6.9)	0.732 (0.011)	0.800 (0.008)	-1.67 (0.15)	-2.56 (0.34)		-2.91 (0.22)	-3.31 (0.28)		42.8 (9.8)	157.3 (9.8)
	combined	69.3 (4.7) B	53.9 (4.6) B	0.770 (0.013) AB	0.774 (0.015) AB	-2.21 (0.20) B	-2.90 (0.24) B		-4.02 (0.46) ns	-4.22 (0.39) ns		53.2 (14.8) A	152.6 (10.3) A

browsed plots of *C. arboreus* when compared at the end of the summer 2008 dry season (Table 3 and Fig. 2; t-test, for site 2 $t = -0.233$, $df = 25$, $P = 0.826$; for site 3, $t = 0.744$, $df = 21$, $P = 0.465$) nor when compared at the end of the summer 2009 dry season (t-test, for site 2, $t = -0.532$, $df = 25$, $P = 0.599$; for site 3, $t = 0.855$, $df = 21$, $P = 0.402$). Although there were significant differences between browsed and non-browsed plots of *C. arboreus* in some physiological traits, these differences were not greater than differences that were present between fenced and non-fenced plots for the other two species where browsing was not occurring (Table 3). Thus, these potential browse differences are difficult to separate from differences due to site heterogeneity. For cross-species comparisons, data from fenced and non-fenced plots have been pooled for most of the remaining results.

Seedling survival declined throughout the dry season, with seedlings at site 4 (*A. catalinae*) exhibiting the steepest decline (Fig. 2). Sites significantly differed in seedling survival following the 2008 summer dry season (Table 3, Fig. 2; ANOVA $F_{3, 121} = 16.063$, $P < 0.001$). The highest survival occurred at site 4, which was dominated by *C. megacarpus* (69.3 ± 4.7 %), followed by *C. arboreus* (50.0 ± 4.7 at site 2 and 62.5 ± 3.7 % at site 3), with the lowest survival at site 1, which was dominated by *A. catalinae* (29.6 ± 4.5 %). The survival of *A. catalinae* at site 1 was significantly lower than for the other three sites that were dominated by the other two species. A similar pattern remained following the second dry season in summer 2009 (Table 3, Fig. 2; Kruskal-Wallis Analysis of Variance on ranks, $df = 3$, $H = 39.987$, $P < 0.001$). In October 2009, both *C. megacarpus* and *C. arboreus* (32.3 ± 4.2 at site 2 and 56.0 ± 4.6 % at site 3) had relatively high survival, and the lowest survival was again at site 1 where *A. catalinae* occurred. The survival of site 1 *A. catalinae* in October 2009 was significantly lower than site 4 *C. megacarpus* and site 3 *C. arboreus*, but was not different from site 2 *C. arboreus*. Lower initial densities combined with lower survival resulted in low post-fire seedlings densities for *A. catalinae* two years post-fire when compared to the other two species (Table 2).

Predawn and midday water potentials, stomatal conductance, and fluorescence differed between species and with season during the first two years post-fire (Table 3, Fig. 3). Of the three species, only *A. catalinae* exhibited a mean dark adapted fluorescence of < 0.75 indicating it was experiencing some photoinhibition. The timing of the photoinhibition coincided with the greatest decline in seedling survival during the 2008 dry season suggesting that stress during the dry period was linked to mortality. During this same time, *A. catalinae* seedlings also displayed the lowest predawn (Kruskal-Wallis Analysis of Variance on ranks, $df = 2$, $H = 13.533$, $P = 0.001$) and midday water potentials (Kruskal-Wallis Analysis of Variance on ranks, $df = 2$, $H = 10.322$, P

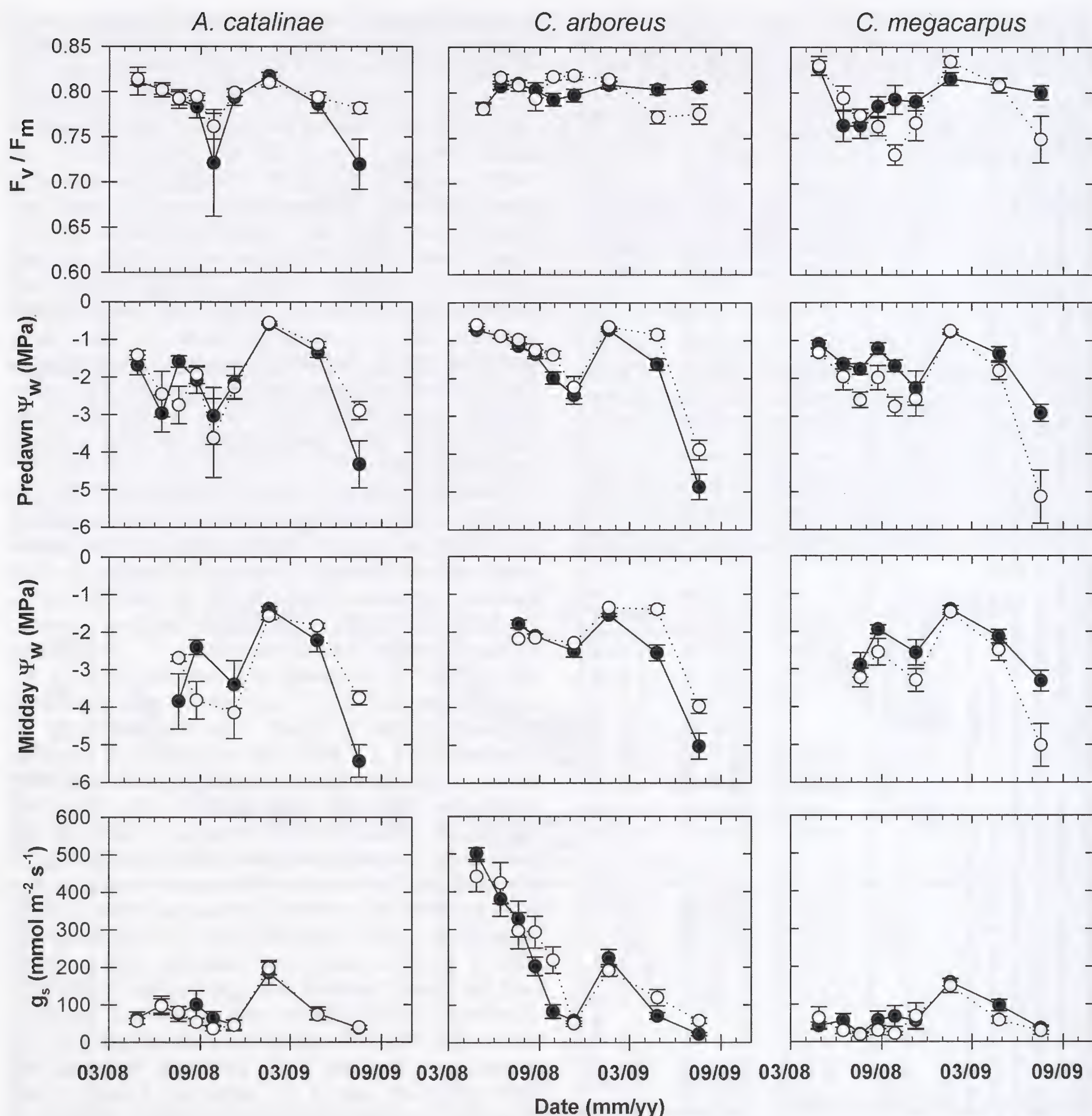


FIG. 3. Dark adapted fluorescence (F_v/F_m), predawn and midday water potentials (Ψ_w), and stomatal conductance of seedlings of three island endemic chaparral species or subspecies during the first two years post-fire during seedling establishment. Open symbols are from plots that were non-fenced and therefore open to potential browse activity and closed symbols are from plots that were protected from browse by tall fences. Symbols represent means ± 1 SE.

= 0.006). We also measured the lowest water potential value of the study in a very dry individual seedling of *A. catalinae* during summer 2008, including an individual with a water potential of -9.82 MPa predawn. Stomatal conductance was generally low for both *A. catalinae* and *C. megacarpus* seedlings and much higher in *C. arboreus* (Table 3, Fig. 3), which mirrored the differences in growth rates among these species (Fig. 2). *Arctostaphylos catalinae* was delayed the first year when compared to the other species, as indicated by a later peak in stomatal conductance, with a June peak in stomatal conductance (i.e.,

maximum seasonal g_s) for *A. catalinae* compared to April for the other two species (Table 3, Fig. 3). Differences among species were less pronounced in the second year post fire for all physiological traits (Table 3, Fig. 3).

Vulnerability to cavitation varied among species, but did not vary with browsing in *C. arboreus*. The P_{50} of browsed versus non-browsed individuals of *C. arboreus* were not different (t-test, $t = 0.738$, $df = 10$, $P = 0.478$); therefore, data from both browsed and non-browsed plants were pooled for comparisons among species. Vulnerability to water-stress induced

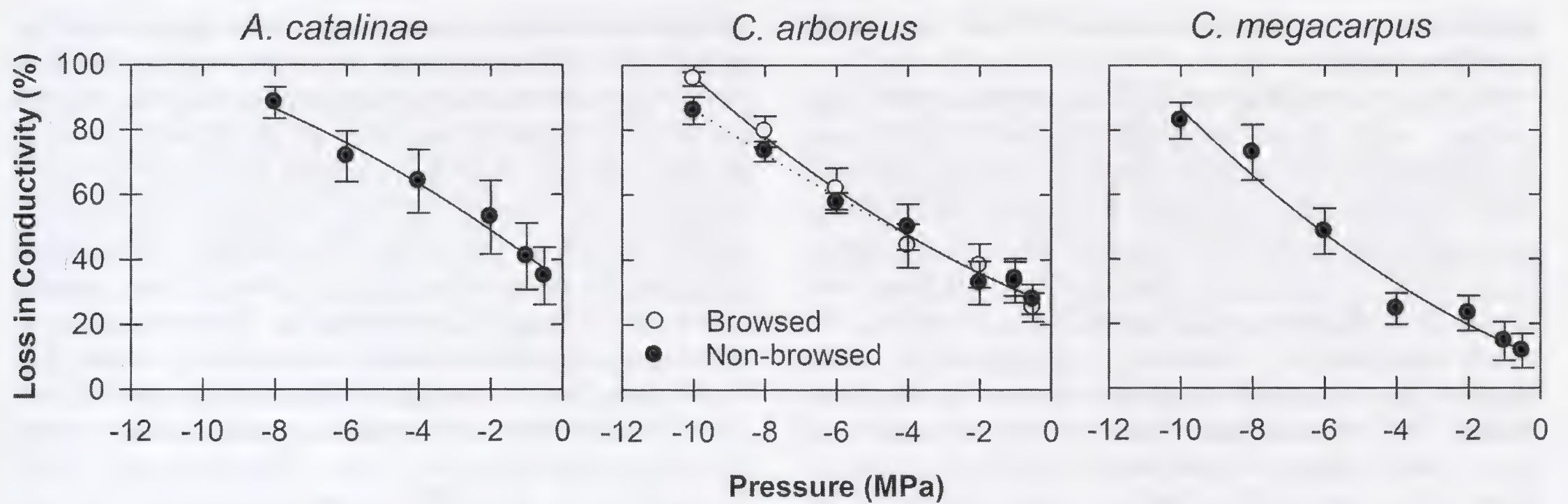


FIG. 4. Vulnerability to cavitation curves of seedlings of three island endemic chaparral species or subspecies. Open symbols are from seedlings that had been browsed and closed symbols are from seedlings that had not been browsed. Symbols represent means \pm 1 SE. No browsed plants were measured from *A. catalinae* and *C. megacarpus*, because seedlings of these species were not being browsed at the time that samples were collected.

cavitation varied among species (Fig. 4; ANOVA, $F_{2,21} = 4.787$, $P = 0.016$). *Arctostaphylos catalinae* was significantly more vulnerable than *C. megacarpus* ($P_{50} = -4.17 \pm 0.62$ compared to -6.74 ± 0.60 MPa, $P < 0.05$) and *C. arboreus* was intermediate ($P_{50} = -6.00 \pm 0.44$) and not different from the other two species ($P > 0.05$).

DISCUSSION

Deer strongly favored *C. arboreus* seedlings and we observed very little browsing of the other two studied species, although deer were active at all sites and there was evidence of browse on co-occurring resprouting shrubs. Browsing activity on *C. arboreus* seedlings varied seasonally, with the greatest levels of browsing occurring during the summer dry season, presumably because of reduced browse options on the landscape. These high levels of deer browse are consistent with the nitrogen fixation capacity of this species and its high rates of growth (Herms and Mattson 1992).

Browsing did not significantly affect seedling mortality; however, browsing did significantly affect plant height, consistent with findings on post-fire seedlings of *Ceanothus* species on the mainland (Thomas and Davis 1989). The other physiological parameters that we measured, including water potential, stomatal conductance, fluorescence, and vulnerability to cavitation were not impacted by browsing. If browsing persists, seedlings will likely not be able to tolerate the high intensity of browsing that we observed in the long term (e.g., Ramirez et al. 2012); moreover, this browsing will dramatically impact stand structure and canopy coverage as stands mature. The effect of browse on carbon balance of plants, including the inability of the plants to develop a full photosynthetic canopy, may also make them more vulnerable to drought (Trifilò et al. 2017) and they will not be able to reproduce, thus no seed bank will form. Monitoring of these plots is ongoing as important plant/deer interactions are

occurring over long time scales. The large differences in canopy coverage between browsed and protected sites will likely affect island ecology (Ramirez et al. 2012), the flowering and fruiting of browsed populations (Dvorak and Catalano 2016), and plant physiological performance (Pittermann et al. 2014).

A. catalinae seedlings were not browsed and *C. megacarpus* experienced only limited browse during late summer 2009. The lack of browse for these species was likely related to two factors. First, the slower growth rates of these species meant that they were much shorter than *C. arboreus* seedlings and most resprouting shrubs as well. These results are consistent with post-fire deer browse of *Ceanothus megacarpus* and *C. spinosus* seedlings on the mainland (Thomas and Davis 1989). Thomas and Davis (1989) found that deer did not browse post-fire seedlings until the seedlings achieved a certain height. The undesirability of some species, particularly of *A. catalinae*, to the deer is likely linked to the small stature of their seedlings. As these plants grew taller, and after the completion of the present study, they later became targets of intense browse (Jacobsen and Pratt, unpublished data). For *C. megacarpus*, browse only occurred toward the end of the second summer when plants had grown substantially (> 40 cm in height). However, this does not fully explain the pattern, since there were some individuals that were above the apparent minimum browse height that were not browsed.

A second possibility may be that *A. catalinae* and *C. megacarpus* are less palatable and therefore are not as preferred as *C. arboreus*, especially during the post-fire period when there are also other abundant herbaceous browse options available. Indeed, preference for *C. arboreus* has been previously described, although this was from a prior period on the island when goats and pigs, as well as deer, were present and potentially impacted vegetation patterns and browse behavior (Knapp 2005). If differential browsing pressure is maintained, it may have important consequences for long-term species abundances and

distributions on Santa Catalina Island for these island endemics.

Seedling survival levels differed between the three species, with most seedling mortality occurring during the first dry season post-fire. This is consistent with what has been described for chaparral seedlings germinating post-fire at mainland sites (Kummerow et al. 1985; Frazer and Davis 1988; Thomas and Davis 1989; Moreno and Oechel 1992). However, the initial densities of post-fire seedlings were much higher than reported for mainland sites (Keeley and Zedler 1978; Frazer and Davis 1988; Thomas and Davis 1989; Moreno and Oechel 1992). Our density values were especially striking when compared as initial ratios of pre-fire adult to post-fire seedling densities. Our values for *Ceanothus* species ranged between 5,218 to 19,617 compared to two mainland studies that ranged between 4.3 to 10.0 (Frazer and Davis 1988; Thomas and Davis 1989). This may be due to the long inter-fire period preceding the fire, which allowed abundant and long-lived seeds to build up in the soil seed bank. It is also likely that seed bank predators are absent in some areas of the island or are at low population densities, which allows large seed banks to persist. Seedling densities remained quite high at the conclusion of the present study. Additional thinning has occurred at the sites and will presumably continue as the stand matures (Hanes 1971; Schlesinger and Gill 1978).

In contrast to the pattern we observed with respect to seedling densities, our sites on the island showed much lower densities of pre-fire adults than those previously reported on the mainland (Schlesinger and Gill 1978; Keely and Zedler 1978; Mills 1986; Frazer and Davis 1988; Thomas and Davis 1989; Moreno and Oechel 1992). This may be because island plants do not live as long as mainland plants, although we are not aware of any prior data supporting this potential explanation. It may also be due to a long fire-free period on the island prior to the current fire, as low adult densities and relatively high seedling to parent ratios have been described for ancient mainland chaparral sites (Keeley et al. 2005). Alternatively, it may be due to impacts from introduced herbivores in the long fire-free period prior to the current study. Although mule deer and bison (*Bison bison* L.) are the only large introduced mammals currently on the island, the island has also hosted introduced cattle (*Bos taurus* L.), sheep (*Ovis aries* L.), goats (*Capra hircus* L.), and pigs (*Sus scrofa* L.) within the last century. Goats and pigs, in particular, were documented to have large impacts on the Santa Catalina Island vegetation, even in the absence of fire (Coblentz 1977; Schuyler et al. 2002; Knapp 2005). In the absence of these large mammalian herbivores, the predicted mature shrub densities for protected plots is thus difficult to estimate, although a range of 0.4 – 6 individuals per m² would be consistent with previous studies from the mainland. *Arctostaphylos catalinae* seedling densities already fall within this range, just two years post-fire.

Differential dry season survival appears to be related to differences in drought tolerance and physiology at the seedling stage when rooting depths are shallow causing seedlings to be especially prone to dehydration. The *A. catalinae* seedlings at site 1 exhibited the lowest survival through the first dry season as well as the second dry season. This species was also the most vulnerable to water-stress induced hydraulic failure and experienced the most negative water potentials. Consistent with these patterns, F_v/F_m values for *A. catalinae* were significantly lower than for the other two species suggesting that plants were experiencing greater stress (Venturas et al. 2016; Guadagno et al. 2017). Lower survival for this species is consistent with a previous study that found that P_{50} was a strong predictor of chaparral seedling dry season survival (Pratt et al. 2008), with the most vulnerable species displaying the highest mortality. This is also consistent with increased water stress of seedlings due to decreased rooting depth and extent compared to resprouts and mature plants (Frazer and Davis 1988; Thomas and Davis 1989; Clemente et al. 2005; Padilla and Pugnaire 2007). Additionally, *A. catalinae* seemed relatively vulnerable to dehydration-induced vascular damage while the *Ceanothus* spp. did not. The P_{50} for the two *Ceanothus* species were similar or only slightly more vulnerable than values reported for mainland adults and seedlings of *Ceanothus* (Jacobsen et al. 2007; Pratt et al. 2007; Pratt et al. 2008; Jacobsen et al. 2014), whereas the P_{50} for *A. catalinae* was more vulnerable than previously reported for 9 *Arctostaphylos* species (Jacobsen et al. 2007; Jacobsen and Pratt 2013). Thus, higher mortality for *A. catalinae* seedlings is consistent with its vulnerability. An important caveat to the present study is that different species were sampled at only one or two sites within the post-fire burn area and, especially for *A. catalinae* seedlings, it may be that they are able to survive in higher numbers in more favorable microsites.

In conclusion, although browsing by introduced mule deer has resulted in large reductions in the height of browsed seedlings, this has not yet resulted in impacts on seedling survival or physiology. Species differ in their physiology, and *A. catalinae*, appears to be the most sensitive to dehydration stress of the three examined species and may be particularly threatened by the occurrence of drought during the post-fire recovery period. This species also began with the lowest seedling densities and experienced the greatest seedling mortality, suggesting that it may be particularly at risk. Longer term monitoring will be required to determine whether browsing by introduced mule deer impacts stand composition during the typical thinning phase that occurs with the transition to stand maturity.

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CONSERVATION GENETICS OF THE ENDANGERED DEL MAR MANZANITA
(*ARCTOSTAPHYLOS GLANDULOSA* SUBSP. *CRASSIFOLIA*) BASED ON RAD
SEQUENCING DATA

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ABSTRACT

Del Mar manzanita (*Arctostaphylos glandulosa* Eastw. subsp. *crassifolia* (Jeps.) P.V. Wells) is a federally listed endangered shrub found in San Diego County, California and Baja California, Mexico. This manzanita forms part of the imperiled southern maritime chaparral of southwestern California and adjacent Baja California, Mexico. Del Mar manzanita is problematic to identify because of morphological intergradation with other subspecies of *A. glandulosa*. Such intergradation could result from biological phenomena, such as gene flow among subspecies. Alternatively, it could be that the current circumscription of the Del Mar manzanita is not correct, and that the morphological characters used to diagnose this subspecies are inaccurate indicators of underlying genetics. This situation leads to problems for conservation planning, where accurate identification of individual plants is essential. Here, we used high-throughput sequencing of restriction-site associated DNA markers (RADseq) to develop single nucleotide polymorphism (SNP) data for a large sample of putative Del Mar manzanita, and a small sample of closely related subspecies of *A. glandulosa*. We analyzed genetic relationships using a total of 65,964 SNPs, with the aim of testing whether morphological traits used to identify Del Mar manzanita are an accurate reflection of underlying genetic patterns. We conclude that vegetative morphology is a poor predictor of genetic patterns, and that the current morphology-based circumscription of Del Mar manzanita is probably in need of some change. However, due to the limited sampling of *A. glandulosa* subspecies in this study, it is not possible to determine the taxonomic limits of Del Mar manzanita using our SNP data.

Key Words: California, DNA, genome, military, polyploid, rare, San Diego County.

Arctostaphylos glandulosa Eastw. subsp. *crassifolia* (Jeps.) P.V. Wells (hereafter referred to by its common name, Del Mar manzanita), is an endangered, burl-forming, tetraploid ($n = 26$) shrub found in coastal chaparral habitats of San Diego County, California, and Baja California, Mexico (Federal Register 1996). Like all subspecies of *A. glandulosa*, Del Mar manzanita can re-sprout from a buried lignotuber following fire, and is therefore considered to be strongly fire-adapted (Wells 1969). While pollination biology research has not been carried out on the Del Mar manzanita specifically, at least one close relative is essentially self-incompatible (Fulton and Carpenter 1978), so Del Mar manzanita is assumed to employ the same strategy.

Del Mar manzanita is generally found on sandstone-derived terraces near the sea (Federal Register 1996; Parker et al. 2012) and is one of several key

indicators of the imperiled southern maritime chaparral plant community (Hogan et al. 1996). Throughout its small geographic range, Del Mar manzanita faces threats from urban development and other human activities, which have led to the loss of many populations and the fragmentation of remaining ones, especially in far western San Diego County (Federal Register 1996; USFWS 2010). Although Del Mar manzanita has been identified in northern Baja California, Mexico (USFWS 2010; Parker et al. 2012), the taxon has no special legal status according to Mexico's list of protected taxa, the Norma Oficial Mexicana (Mexican Official Standard) NOM-059 (SEMARNAP 2002).

Along the eastern margin of its geographic range, Del Mar manzanita comes into contact with its close relative, Eastwood manzanita (*A. glandulosa* Eastw. subsp. *glandulosa*; including *A. glandulosa* Eastw.

TABLE 1. COLLECTING LOCALES AND SAMPLING INFORMATION. *Taxon* represents *A. glandulosa* individuals identified according to dichotomous keys in the Jepson Manual (Parker et al. 2012). *Code* indicates a field collection code for each locale used as a short-hand to refer to individual locales; each code corresponds to an herbarium voucher specimen housed at the UC Davis Center for Plant Diversity or the San Diego Natural History Museum (only one herbarium voucher was collected per site). M. Mulligan sampled locales 3318 and 3321; D. Burge sampled all other locales. *Samples* correspond to the number of individuals sequenced. Latitude and longitude are given in the WGS 84 datum.

Taxon	Code	Locale name	Samples	Latitude	Longitude
<i>A. g. ssp. atumescens</i> <i>A. g. ssp. crassifolia</i>	2006	Cerro Buenavista	1	31.6737	-116.6314
	1694	Cerro del Coronel	1	32.2830	-116.9300
	1701	Cañon San Isidro	2	31.2920	-116.3434
	1703	Mesa de Descanso	4	32.1718	-116.8898
	1709	Encinitas Community Center	3	33.0444	-117.2669
	1717	Torrey Pines	3	32.9406	-117.2471
	1719	MCAS 1	2	32.9164	-117.0398
	1720	MCAS 2	3	32.8897	-117.0640
	1722	MCAS 3	3	32.8787	-117.0659
	1723	MCAS 4	3	32.8649	-117.0690
	1725	MCAS 5	3	32.8932	-117.0757
	1729	Crest Canyon	4	32.9501	-117.2538
	2071	Encinitas	3	33.0360	-117.2487
	3318	MCAS 18	3	32.8938	-117.0398
	3321	MCAS 17	3	32.8915	-117.0516
<i>A. g. ssp. glandulosa</i>	1523	Cavedale Road	2	38.3624	-122.4719
	1688	Newhall Pass	3	34.3471	-118.5102
	1746	Mount Tamalpais	4	37.9110	-122.5775
	2033	Bolinas Ridge	2	37.9468	-122.6665
	2087	Viejas Mountain	1	32.8540	-116.7421
	2090	Santa Ana Mountains	1	33.6535	-117.4455

subsp. *zacaensis* Eastw.; Parker et al. 2012). Eastwood manzanita is a plant of interior habitats in the Coast and Peninsular Ranges from central California to northern Baja California, Mexico. Although the extent to which genetic exchange among wild populations takes place is not known , the close geographic proximity of the two subspecies in San Diego County suggests that there are opportunities for such exchange (Keeley et al. 2007).

Like many *Arctostaphylos*, Del Mar manzanita is difficult to identify, with morphological traits used to define this taxon intergrading into other subspecies of *A. glandulosa*, particularly Eastwood manzanita. In the past, this continuity was assumed to result from gene flow among taxa (Keeley et al. 2007). However, it is also possible that the morphological traits used to define taxa are simply poor indicators of underlying genetic patterns. In the latter case, new circumscriptions may be required to bring taxonomy into line with biology. As with any rare plant, taxonomic ambiguity and resulting problems with identification can lead to difficulties for conservation and recovery. Therefore, tools are needed that facilitate objective means of identification. Genetic analyses provide such a tool (Ogden et al. 2009; Kelly 2011). In the Del Mar manzanita and its closest relatives, combined genetic and morphometric analysis could reveal whether circumscriptions based on morphology are reflected by genetic patterns.

We employed restriction site associated DNA (RAD) sequencing (Miller et al. 2007; Davey and Blaxter 2010) to develop a large single nucleotide polymorphism (SNP) dataset for putative populations of Del Mar manzanita (including its type locality in the city of Del Mar, San Diego County) and a sample of its close relatives, especially those found in southern California and northern Baja California, Mexico. In recent years, RAD sequencing has become a common strategy to obtain genome-level information on genetic variants for use in both animal and plant conservation genetics (Allendorf et al. 2010; Hohenlohe et al. 2011; Lozier 2014; Jennings et al. 2016; Torres-Martínez and Emery 2016; Lozier and Zayed 2017).

We also collected vegetative morphometric data from leaves and stems to compare to genetic patterns. We then analyzed the morphological and genetic data, with the aim of testing whether morphological traits used to circumscribe Del Mar manzanita match genetic patterns. Overall, the results of this study will provide a genetic perspective for future management and conservation work on the Del Mar manzanita, and could have implications for the conservation, taxonomy, and systematics of other manzanitas.

MATERIALS AND METHODS

Taxon Sampling

For sampling of the Del Mar manzanita, we visited 14 locations in California and Baja California, Mexico (Table 1, Fig. 1, Appendix 1). In some large populations, we sampled from several subpopula-

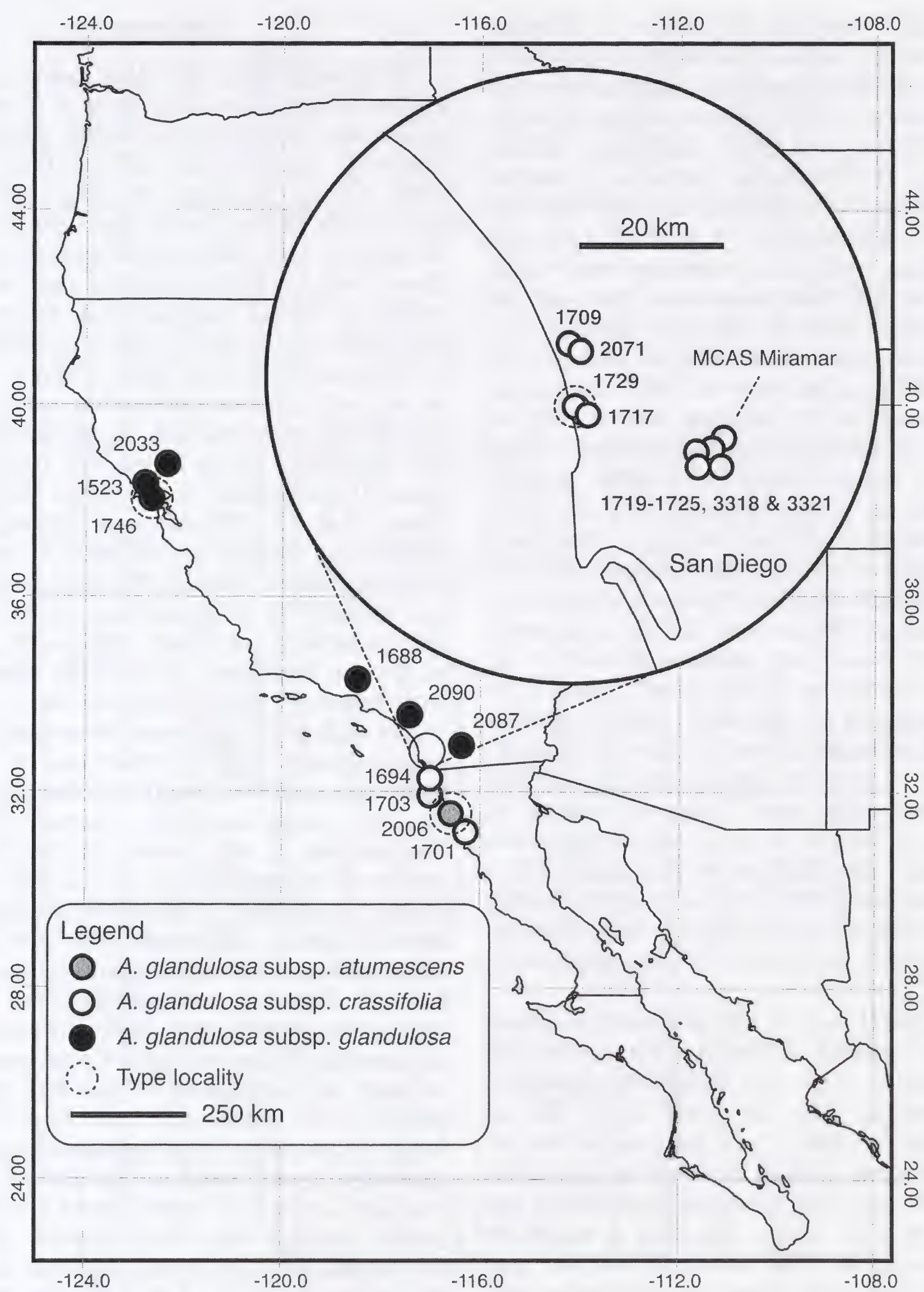


FIG. 1. Sampling map. Grid dimensions are in degrees of latitude and longitude (WGS84 datum). See Table 1 for more information on locales.

tions. To prevent confusion between populations and subpopulations, we refer to plant collecting locations as *locales*, which we define as groups of plants that are discontinuous from one another, with centers at least one km apart. In every case, the edges of the locales were at least 0.5 km apart. We were not able to obtain estimates of population size. However, the spatial density and size of populations/subpopulations vary dramatically across the range of the Del Mar manzanita (AECOM 2015), with consequences for population genetics.

We sampled intensively in central San Diego County, especially at the Marine Corps Air Station

(MCAS) Miramar, a federally owned property managed by the United States Department of Defense (Fig. 1). MCAS Miramar is thought to contain large populations of Del Mar manzanita, and is also a zone of potential contact between this taxon and Eastwood manzanita (Rebman and Dossey 2002).

To aid in determining the correspondence of genetics with morphometric taxon concepts, we sampled from the taxon thought to be most closely related to Del Mar manzanita, the Eastwood manzanita (six locales, Table 1, Fig. 1), and from *Arctostaphylos glandulosa* subsp. *atumescens*

J.E.Keeley, M.C.Vasey & V.T.Parker, a Mexican endemic that is the only subspecies of *A. glandulosa* other than the Del Mar manzanita and the Eastwood manzanita known to occur close to the coast in southern California and Baja California, Mexico (Table 1, Fig. 1). This latter species is a narrow endemic found only on Punta Banda Peninsula in Baja California, Mexico (Fig. 1), where it is the only manzanita. Thus, while this species does occur in the same region as Del Mar manzanita, they are not known or expected to come into close contact.

Here we use scientific names that are based on the morphology of the plants prior to DNA sequencing. Because the purpose of our study was to find out whether plants identified as Del Mar manzanita using morphological criteria could be reliably grouped together based on genetic similarity, we began by using names based on the morphology of the plants that we sampled (Table 1). Plants were identified according to the taxonomic keys and descriptions in the Jepson Manual (Parker et al. 2012), supplemented by relevant taxonomic revisions (Keeley et al. 2007). In both Parker et al. (2012) and Keeley et al. (2007), the presence of glandular hairs on leaves, twigs, and inflorescences, as well as color of the leaves and shape of the fruit are used to discern Del Mar manzanita from other taxa. Using this means of identification allowed us to begin with a hypothesis as to the correct identification of the plants, which was then tested using DNA. In the results presented below, we call attention to groups of plants in which the DNA results do not agree with the morphology-based names.

We took special care to sample multiple plants from the type localities of Del Mar manzanita (Del Mar, CA; Jepson 1922) and Eastwood manzanita (Mount Tamalpais, CA; Eastwood 1897). We included the type localities in the analysis so that we could make progress toward establishing the genetic limits of these taxa. We recognize that this is not ideal in the eyes of some, and that it might be preferable to sample DNA directly from the type specimens for such work. However, preliminary tests showed that DNA is not recoverable from older herbarium specimens in sufficient quantities to allow RAD DNA sequencing, the genomic method employed here. Therefore, sampling living plants at the type localities, rather than from the type specimens themselves, represents a next-best approach. This may also have the advantage of identifying relevant variation in morphology and genetics currently present in the population of the type locality.

For all sampled plants, we collected young leaves and flower buds, which were frozen on dry ice within 48 hours of collection. Up to 10 individuals were sampled from each locale, but not all collected individuals were sequenced (Table 1). We collected more individuals than required due to the low rate of successful DNA isolation; only high-quality samples were used.

Morphometrics

Morphologically, Del Mar manzanita is distinguished from other subspecies of *A. glandulosa* by qualitative characteristics of the reproductive and vegetative parts (Parker et al. 2012; Keeley et al. 2007; Table 2), many of which are subjective, particularly in the case of leaf color. Because most of the sites were visited in the spring, to obtain fresh flower buds for DNA extraction (see below), we were unable to collect material that included fruit and were therefore unable to use fruit characters in our morphometric analysis. We also did not collect data on leaf color. Though leaf color is often used to help distinguish subspecies of *A. glandulosa* from one another (Table 2), we found that it was difficult to assess this character in our dried herbarium specimens, due to differences in drying conditions. Instead, we focused on the hairs of the leaves and youngest stems (Table 2). If present at all, these hairs are typically in two layers, one of longer, stiff hairs that sometimes are tipped with glands, and another of shorter hairs that are typically appressed to the stem below the level of the long hairs.

For each of the specimens from which we obtained genetic data, we also collected data on the following traits: (A) *long hair density*, the density of long hairs on the young stem (0, absent; 1, sparse; 2, intermediate; 3, very dense), (B) *long hair type*, the texture of the long hairs (1, stiff; 2, soft), (C) *long hair glands*, the presence of glands at the tips of the long hairs (0, absent; 1, present), (D) *short hair presence*, the presence of short hairs below the layer of the long hairs (0, absent; 1, present), (E) *short hair type*, the type of the short hairs (1, stiff and erect; 2, soft and appressed), (F) *margin hairs*, the presence or absence of hairs on the margin of the leaf, (0, absent; 1, present), (G) *margin hair type*, type of the margin hairs, if present (1, long, glandular; 2, short, nonglandular; 3, stiff, long, nonglandular), (H) *quantitative leaf shape* (leaf length (mm) divided by width (mm); average for three mature leaves). Raw morphological data were deposited at the Dryad Digital Repository (Appendix S1; doi: 10.5061/dryad.kv573c7).

The vegetative morphometric data were treated in a multivariate framework. We visualized data using principal components analysis (PCA) and tree reconstruction, carried out in R, version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria). For PCA, we used the *prcomp* function in R to model the seven categorical variables and the single quantitative variable (H, leaf shape). Quantitative leaf shape was transformed into a Z-score before analysis. Also, due to the needs of the model, all individual plants with “missing” data (cases in which characters were not applicable to the individual in question) were excluded from analysis. The first two principal components were visualized in bivariate space to examine relationships. The contribution of each morphological character to the

TABLE 2. KEY MORPHOLOGICAL TRAITS TRADITIONALLY USED TO DISTINGUISH SUBSPECIES OF *A. GLANDULOSA*. Traits are taken from Keeley et al. (2007) and Parker et al. (2012).

Taxon	Leaf color	Leaf vestiture	Nascent inflorescence shape	Nascent inflorescence bract vestiture	Fruit shape	Basal burl	Branchlet vestiture
<i>A. g. ssp. adamsii</i>	Strongly white-glaucous	Glabrous	Pendant	Non-glandular	Spheric to slightly depressed spheric	Present	Short-nonglandular
<i>A. g. ssp. atumescens</i>	Green to gray-green	Glabrous	Pendant	Glandular	Spheric to slightly depressed spheric	Absent	Long-glandular
<i>A. g. ssp. crassifolia</i>	Dark green	Glabrous to pubescent	Pendant	Non-glandular	Strongly depressed-spheric	Present	Short-nonglandular
<i>A. g. ssp. cushingiana</i>	Green to gray-green	Glabrous	Pendant	Non-glandular	Spheric to slightly depressed spheric	Present	Short-nonglandular
<i>A. g. ssp. erecta</i>	Yellowish green	Pubescent	Erect	Glandular	Spheric to slightly depressed spheric	Present	Short-nonglandular
<i>A. g. ssp. gabrielensis</i>	Lustrous green	Glabrous	Pendant	Non-glandular	Spheric	Present	Short-nonglandular
<i>A. g. ssp. glandulosa</i>	Green to gray-green	Glandular-puberulent	Pendant	Glandular	Spheric to slightly depressed spheric	Present	Both short- & long-glandular
<i>A. g. ssp. howellii</i>	Green to gray-green	Glandular to nonglandular	Pendant	Glandular	Spheric to slightly depressed spheric	Present	Short-nonglandular
<i>A. g. ssp. leucophylla</i>	Strongly white-glaucous	Glandular to puberulent	Pendant	Glandular	Spheric to slightly depressed spheric	Present	Both short- & long-nonglandular
<i>A. g. ssp. mollis</i>	Bright green	Glabrous	Pendant	Non-glandular	Spheric to slightly depressed spheric	Present	Both short- & long-nonglandular

principal components was determined based on vector loadings. Based on preliminary results of the PCA analysis, it was determined that only A, C, D, and G contributed significantly to the model (absolute value of maximum vector loading on first and second principal component < 0.09).

Based on the seven categorical variables, we constructed a classification tree using the *rpart* R package, version 4.1-10. The *rpart* package implements recursive partitioning (Therneau and Atkinson 1997; De'ath 2002). In recursive partitioning, a tree linking all of the observations (individual plants in this case) is built by a simple process: a categorical variable is selected that “best” splits the individuals into two groups. This process is applied until the subgroups either reach a minimum size, or all individuals are grouped. We set the “minsplit” parameter of *rpart* to 2, the “minbucket” parameter of *rpart* to 1, and the “cp” parameter to 0.001. We did not prune the resulting tree, because cross-validation suggested that no improvement could be made.

In addition to recursive partitioning, we constructed a distance-based tree to visualize relationships among individual plants based on their overall dissimilarity across the categorical variables that we scored. We constructed a UPGMA (Unweighted Pair Group Method with Arithmetic Mean) tree using the *vegan* package of R, version 2.3-4. We used the *hclust* function of *vegan*, with ‘method’ set to average. Based on the preliminary results of the PCA, we excluded traits that did not contribute strongly to the preliminary PCA analysis, as these would likely add noise to the UPGMA tree; the UPGMA tree used traits A, C, D and G (see above).

DNA Sequencing & Variant Detection

Total genomic DNA was extracted from flower buds using the DNeasy Plant Mini Kit (Qiagen, Germantown, MD) according to the manufacturer's instructions. Total genomic DNA was checked for degradation on a 1% agarose electrophoresis gel; samples with high molecular-weight DNA (~ 20 – 50 kb) were standardized to 30 ng/ μ L (diluted to a volume of 150 μ L in TE) and sent for quality control, library construction, and sequencing at Floragenex, Inc. (Portland, OR). Methods for this process generally followed those described by Lozier (2014), with the exception that our work used the restriction enzyme PstI (Hipp et al. 2014). In brief, sequence identifier barcodes (a unique one for each plant) and sequence adapters were added to genomic DNA after digestion by the endonuclease PstI. The barcoded samples were then combined and the fragments sequenced outwards from restriction-sites using single-end DNA sequencing reads that were 100 base pairs long. All DNA sequencing was done on a HiSeq 2500 DNA sequencing instrument (Illumina, San Diego, CA). Following sequencing, DNA sequences from individual plants were separated

based on their unique barcodes using the program *fastq-multx* (Aronesty 2013); the barcodes were then removed from the sequences.

Due to the lack of an existing reference genome for *Arctostaphylos* or a closely related member of the Ericaceae, detection of variation among individual plants was done using a “RAD reference” approach (Lozier 2014), in which a kind of reference genome is constructed using sequence data from the plant with the greatest number of unique RAD clusters. We employed this strategy to ensure the best reference genome “target” for subsequent alignment of sequences from other plants. In developing the RAD reference, custom methods developed by Floragenex (Lozier 2014) were used to cluster identical sequences that had 5 – $500\times$ sequencing coverage, which produced a preliminary assembly. The assembly was then collapsed back to separate sequences and these sequences were realigned against the preliminary RAD reference genome using the program BWA [Li and Durbin 2009; *aln function, edit distance* ($-n$) 3 , $-N$ (*disable iterative search*)], allowing at most four mismatches among reads within a cluster. The purpose of self-alignment was to identify and remove repetitive DNA regions.

Reads for each individual plant were aligned to the RAD reference using BOWTIE (Langmead et al. 2009), relying on sequence quality information to aid in the process of match-making, allowing a maximum of three mismatched bases per read, and permitting alignment of each read to no more than one region of the reference. SAMTOOLS (Li et al. 2009) was used to detect SNPs and call genotypes. Filters for SNP calling required a minimum phred score of 20, a minimum of $15\times$ sequence coverage and a maximum of 10% missing data across samples.

Following SNP calling, we also excluded: 1) all positions in which data were missing for any individual plant (Arnold et al. 2013; Davey et al. 2013), 2) all invariant sites (where every individual had the same allele call), and 3) all sites in which more than two allelic variants were detected. The final filtering step restricted the dataset to only biallelic SNPs, thus rendering the data diploid. We used this filter because most population genetic software are not able to deal with polyploid data (where more than two allelic variants are allowed per locus). By rendering our data as biallelic SNPs, we are able to apply population genetic methods that assume diploid loci. We recognize that this is not ideal, as the underlying genetics of the organisms is tetraploid. We think that this filtering method is the best option given the limitations of available SNP calling methods. We also note that this method of dealing with polyploids in population genetic analyses has been employed in other studies (Qi et al. 2015; reviewed by Dufresne et al. 2014). The rendering of loci as diploid when the underlying genetics is polyploid can lead to conflicts with assumptions of software, mainly due to the issue of calculating allele dosage in polyploids (Dufresne et

al. 2014). Methods are available to deal with polyploids in genomic data by calling alleles using a draft reference genome (Garrison and Marth 2012). Unfortunately, the lack of a manzanita reference genome makes it impossible to apply such methods at this time.

The data resulting from the above filtering processes was used to create two datasets, one using all of the *A. glandulosa* samples (54 individuals; Table 1), and the second focused on just the locales classified as Del Mar manzanita, and for which we had more than one sample (39 individuals; Table 1). Hereafter, we refer to the former as the “Complete” and to the latter as the “Reduced” dataset. In both datasets, we excluded invariant sites, as well as sites with a minor allele frequency of less than 0.02 (Bradbury et al. 2007).

Population Genetics

To objectively identify statistically meaningful groups of individuals, we used a combination of parametric and non-parametric approaches. These approaches were applied in exactly the same way to all datasets. For the non-parametric approach, we carried out principal coordinates analysis (PCoA) using an identity by site genetic distance matrix. For the parametric approach, we used the program STRUCTURE version 2.3.4 (Pritchard et al. 2000; Falush et al. 2003, 2007). For each STRUCTURE run, we used 50,000 Markov chain Monte Carlo replicates, applying an admixture ancestry model and assuming diploid loci. In each of the runs, the first 10,000 replicates were discarded as “burnin”, a method that reduces noise in the final sample by excluding samples that were taken while the model was still unstable (Hubisz et al. 2009). For each dataset, we did 10 replicate runs at each level of K from one to thirteen. The appropriate K for each dataset was inferred using the software program STRUCTURE HARVESTER (Earl and vonHoldt 2012), according to the method of Evanno et al. (2005).

Morphology Versus Genetics

To test whether genetic patterns match groupings of plants based on morphology, we used a Mantel test, carried out in the R package *vegan* (version 2.3-4). For the genetic data, we used the same identity by state distance matrix used for PCoA (see above). For the morphological data, we created a Euclidean distance matrix using the eight traits used for the morphometric analyses described above. We ran a partial Mantel test using both Pearson and Spearman correlation coefficients, and controlling for geographic distance.

RESULTS

Vegetative Morphometric Patterns

Patterns of morphological variation across subspecies, locales, and individuals were complex, as

suggested by visualizations of multivariate analysis (Fig. 2A). In PCA, preliminary analysis suggested that only four variables contributed strongly to the model (margin hair type, short hair type, long hair density, long hair glands), and so only these variables were retained for the final analysis. In the final model (Fig. 2A), the first two principal components (those plotted) account for more than 84% of the variance in the data. Long hair density is strongly negatively correlated with the first principal component (vector loading: -0.93), followed closely by margin hair type, which is positively correlated (vector loading: 0.32). Long hair glands and margin hair type both contribute very strongly to the second principal component, but in opposite directions (vector loadings of 0.62 and -0.68, respectively).

The classification tree (Fig. 2B) suggests some of the same patterns as the PCA, indicating that a large number of putative Del Mar manzanita individuals, including some from the type locality, have a lower density of long hairs than other sampled plants. As in the PCA, other sampled individuals are also differentiated by margin hair type and short hair presence; with the exception of long hair glands, the most informative characters are the same in both the PCA and the classification tree.

The UPGMA tree (Fig. 2C) allows for examination of relationships among individual plants. This tree shows that most groupings of individual plants do not conform to expectations based on the morphology of the plants, or even the locality where they were collected (Table 1). Subspecies do not form cohesive groups at any level of the tree, and in many cases individuals from the same locale are not cohesive. Finally, collections from the type locality of Del Mar manzanita are not strongly cohesive and are found in multiple portions of the tree. Overall, these results demonstrate the morphological heterogeneity of the sample, with morphologically distinct plants co-occurring at the same locale.

Genetic Variants

A total of 54 samples were successfully sequenced (Table 1). Quality was high for all samples; none were excluded due to low DNA sequencing coverage or excessive missing data. Sequencing rates averaged 6,903,818 reads per individual (SD = 3,184,037). For information on sequencing coverage for each plant, see Appendix S2 (Dryad Digital Repository). Raw DNA sequence data is on the NCBI BioProject database under accession PRJNA396085.

A total of 16,083,660 reads were available for the sample used to construct the RAD reference genome (D. Burge 2071_4, *A. glandulosa* subsp. *crassifolia*, Table 1). 15,606,332 reads passed quality filters and were assembled into 25,321 contigs 92 of bp in length, for an assembled RAD reference genome length of 2,329,532 bp.

Using the RAD reference genome as a guide, we called a total of 163,793 loci. The complete variant

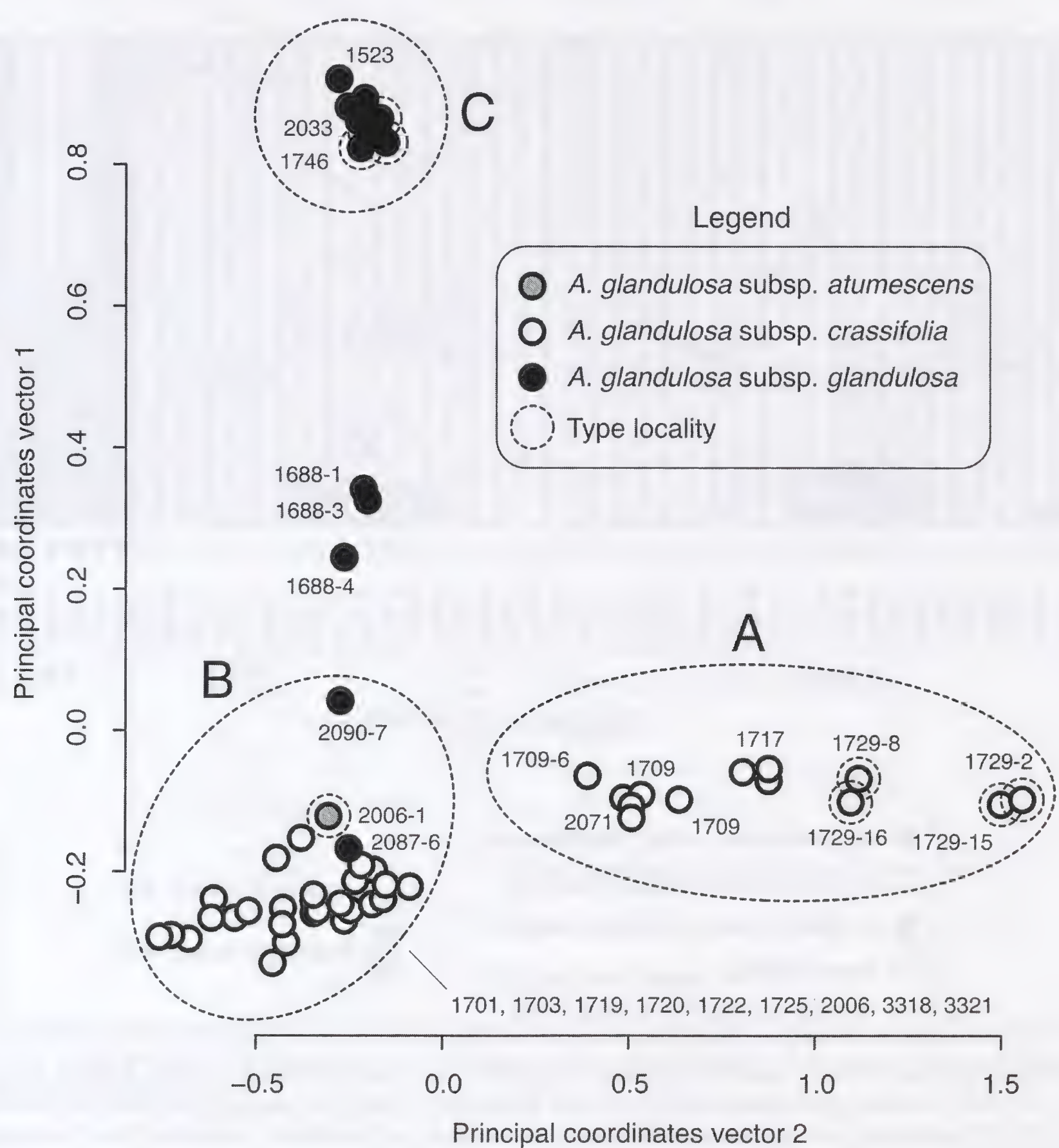


FIG. 3. Genetic relationships based on principal coordinates analysis (PCoA) of all SNP data. Individuals from the type locality of Del Mar manzanita (1729; Del Mar) are indicated with a dashed halo. For clarity of presentation, some of the plants are not labeled individually.

Population Genetics

Principal coordinates analysis of the Complete dataset (Fig. 3) revealed several distinct groups of plants, including: 1) a group comprising all the putative Del Mar manzanitas collected at the type locality, plus several nearby locales (Fig. 3A), 2) a group comprising all remaining samples of putative Del Mar manzanita, plus two individuals of Eastwood manzanita and one of *A. glandulosa* subsp. *atumescens* (Fig. 3B), and 3) a group containing most of the remaining Eastwood manzanitas collected in northern California (Fig. 3C). STRUCTURE analysis of the Complete dataset revealed that the most optimal number of genetic groups was two ($K = 2$). The STRUCTURE HARVESTER output demonstrating this result is available at the Dryad Digital Repository (doi: 10.5061/dryad.kv573c7; Appendix S6). The ancestry proportions inferred by STRUCTURE (Fig. 4) reveal that most of the plants identified as Del Mar manzanita are dominated by one genetic group (Fig. 4, Ancestral Group AG1), while most of the plants identified as Eastwood

manzanita are dominated by the second genetic group (Fig. 4, Ancestral Group AG2). Results of the STRUCTURE analysis also suggests some genetic admixture between these groups in the case of ten plants (Fig. 4, 2006-1, 1709-6, 1717-4, 1717-5, 1717-9, 1688-1, 1688-3, 1688-4, 2087-6, & 2090-7). All of these plants are from MCAS Miramar. PCoA of the Reduced dataset (Fig. 5) revealed groups that match those revealed by the Complete dataset, including: 1) a group comprising all the putative Del Mar manzanitas collected at the type locality, plus several nearby locales (Fig. 5A), and 2) a set of three weakly supported groups comprising all remaining samples of putative Del Mar manzanita (Fig. 5B). STRUCTURE analysis of the Reduced dataset revealed that the most optimal number of genetic groups was two ($K = 2$). The STRUCTURE HARVESTER output demonstrating this result is available at the Dryad Digital Repository (doi: 10.5061/dryad.kv573c7; Appendix S7). The ancestry proportions inferred by STRUCTURE (Fig. 6) reveal that putative Del Mar manzanita individuals come from two fairly distinct genetic groups (Fig. 6,

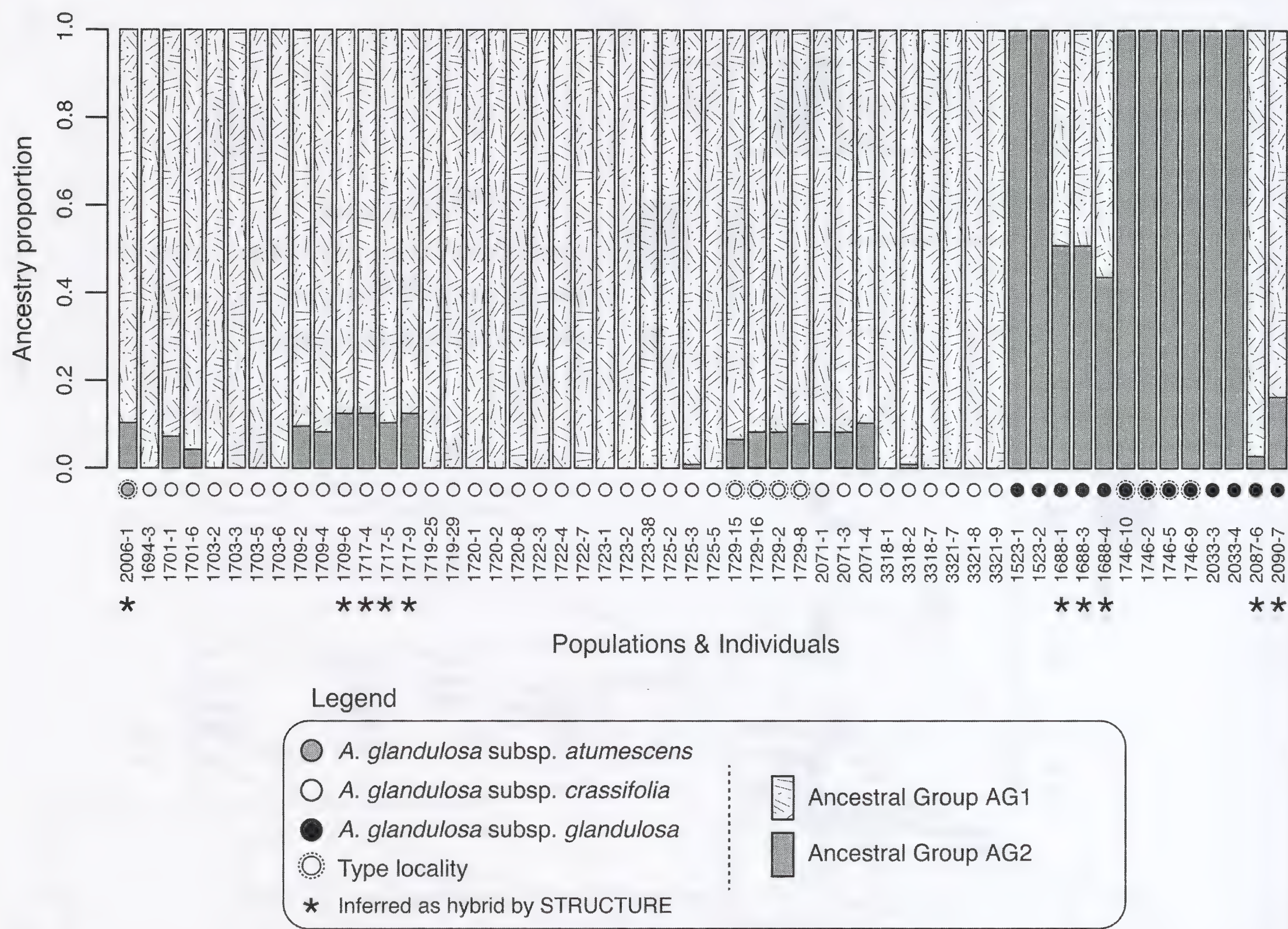


FIG. 4. Proportional genetic ancestry based on analysis of all SNP data using STRUCTURE. Results of analysis with the program STRUCTURE, assuming two ancestral groups (see Methods). Under the assumption that two ancestral groups are present, STRUCTURE estimated the proportion of genetic variation in each sampled individual that was assignable to each of these groups. Proportions are indicated by shaded columns.

Ancestral Groups AGC1 and 2). STRUCTURE results also suggest some genetic admixture between these ancestral groups, represented by six plants that were inferred to be of hybrid origin ($P \ll 0.01$; Fig. 4, 1719-25, 1719-29, 1722-3, 1722-4, 1722-7, and 1725-3). All of these plants are from MCAS Miramar.

Genetics Versus Morphology

The Mantel test using the Pearson correlation coefficient indicated a significant relationship between morphology and genetics (Mantel statistic $r = 0.094$; $P = 0.005$), as did the test using the Spearman correlation coefficient (Mantel statistic $r = 0.069$; $P = 0.022$).

DISCUSSION

Does Morphology Predict Genetic Relationships?

Overall, our Mantel test results suggest that morphology is a reliable predictor of the underlying genetic groups. However, there is a very weak association between genetic groups and the names

that were assigned to the sampled plants based on current morphological circumscriptions of *A. glandulosa* subspecies (Figs. 3 and 4). This lack of correspondence suggests that the current circumscriptions should be modified. Unfortunately, our sampling of *A. glandulosa* subspecies and populations is not broad enough to provide the evidence necessary to support such changes. Future studies should aim to expand on the sampling employed in this study, ideally including a geographically broad sample of *A. glandulosa* populations from all of the known subspecies.

Our morphological analyses did not use fruit characters, due to the time of year in which we sampled. Fruit and seed characters are frequently used in manzanita systematics, and some putative Del Mar manzanita populations are known to have a distinctive (within *A. glandulosa*), flattened fruit (Parker et al. 2012), especially near the type locality, close to the sea (V. T. Parker, San Francisco State University, personal observation). Future research should aim to measure a broader suite of characters than those analyzed in this study, especially those relating to fruit and inflorescence morphology.

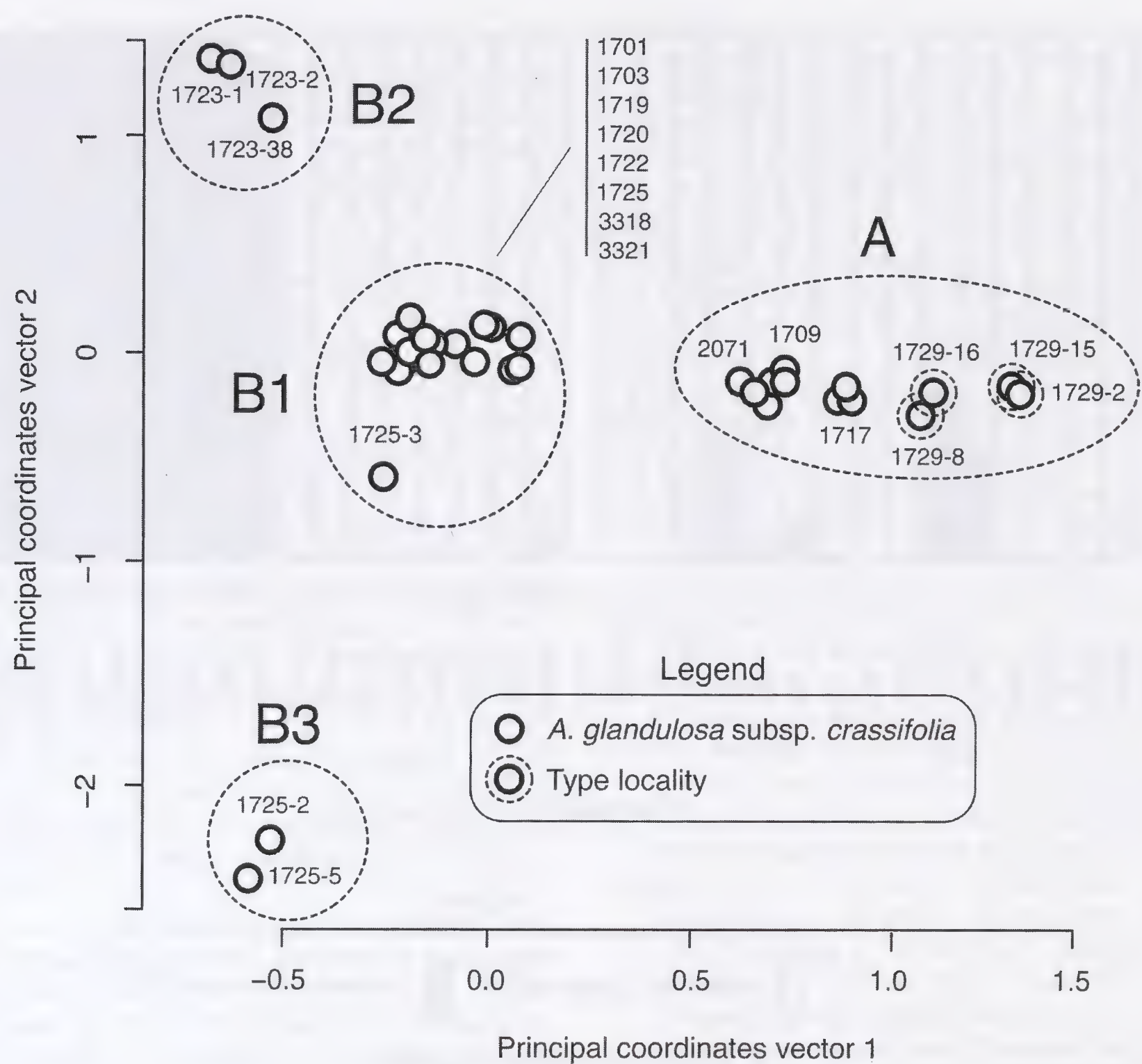


FIG. 5. Genetic relationships based on PCoA of SNP data for only putative Del Mar manzanita. Individuals from the type locality of Del Mar manzanita (1729; Del Mar) are indicated with a dashed halo. For clarity of presentation, some of the plants are not labeled individually.

How Widespread is Del Mar Manzanita?

At the broadest scale, our genetic data suggest that the Del Mar manzanita forms part of a very widespread genetic lineage (Fig. 4, AG1) found mainly in southern California and northern Baja California, Mexico. This result argues in favor of a greatly expanded circumscription of Del Mar manzanita. In this case, Del Mar manzanita would be the dominant burl-forming manzanita in coastal portions of southern California and northern Baja California, Mexico (Fig. 4, AG1). On the other hand, the more focused analysis of only the samples from coastal San Diego County suggests that plants from the type locality and nearby locations close to the coast fall into one genetic group (Fig. 6, AGC1), while plants from more distant locations, especially more inland locations like MCAS Miramar, do not form part of this group. This result suggests that a more restrictive view of Del Mar manzanita might be possible; the circumscription could be modified to include only those plants from the second analysis that fall into the same genetic group as the plants from the type locality (Fig. 6, AGC1), which are from sandstone derived soils very close to the sea. Unfortunately, we did not obtain a broad enough sample of *A. glandulosa* subspecies and populations

to reliably test taxonomic concepts in the group based on DNA, and so we are not able to modify circumscriptions according to genetic patterns. More research is needed to determine taxonomic limits in the subspecies of *A. glandulosa*. Ideally, future research will expand on the present work by adding samples of more subspecies and populations from especially from coastal San Diego County and northern Baja California, Mexico.

Conservation and Recovery Implications

Management of rare plants demands consistent, objective tools to identify these plants to the exclusion of other taxa. Identification is usually done using morphological features. However, many rare plants are difficult to identify morphologically, or only display their diagnostic features during a particular phenological period (e.g., during flowering or fruiting). There are a variety of methods available that seek to overcome this limitation using genetic markers (reviewed by Hollingsworth et al. 2016). However, many of these techniques, particularly DNA barcoding methods (Kress et al. 2005), fall short in groups with low levels of genetic variation. This situation limits objective identification of rare plants, which in turn limits management, where

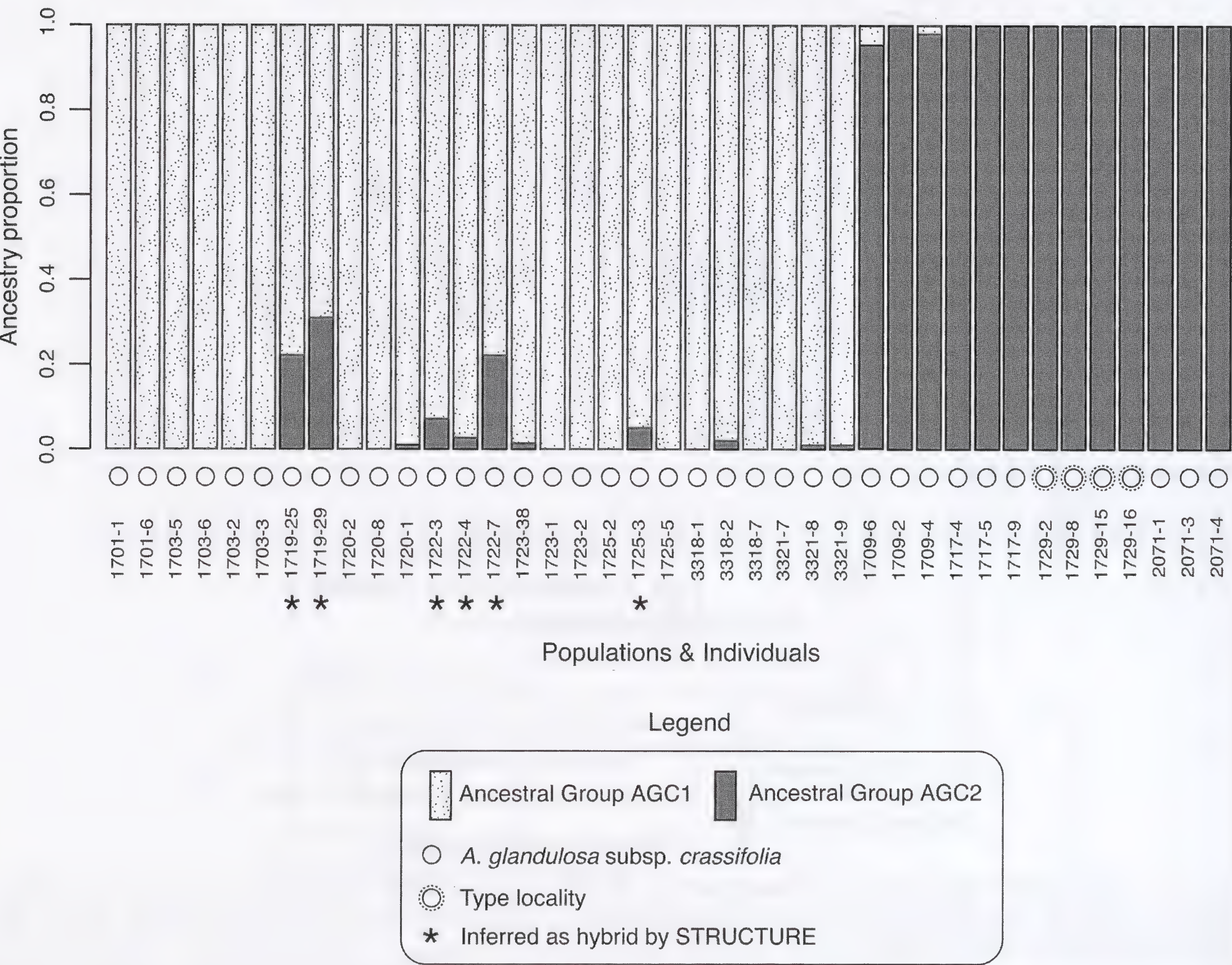


FIG. 6. Proportional genetic ancestry for putative Del Mar manzanitas based on STRUCTURE. Results of analysis with the program STRUCTURE, assuming two ancestral groups (see Methods). Under the assumption that two ancestral groups are present, STRUCTURE estimated the proportion of genetic variation in each sampled individual that was assignable to each of these groups. Proportions are indicated by shaded columns. Individuals marked with an asterisk are considered to be meaningfully admixed at the $P < 0.05$ threshold.

precise identification of rare plants is critical for effective conservation. As demonstrated here and in other recent work (Hollingsworth et al. 2016; Andrews et al. 2016), high-throughput DNA sequencing, particularly RADseq, can be used to target large numbers of variable SNPs without any prior knowledge of the SNPs or the genome of the target organism, allowing for the rapid development of useful panels of genetic markers for precise and repeatable conservation work.

Our results show that in the case of Del Mar manzanita and its close relatives, there is a mismatch between genetic groups and the groups based on current taxonomic concepts. As we explained above, our genetic results could be used equally effectively to argue in favor of either a greatly reduced or a greatly expanded circumscription of Del Mar manzanita. Clearly, these two alternatives have dramatically different implications for conservation and recovery; if we were to choose the first option, this already rare

taxon would become even more rare; if we were to choose the second option, the taxon would become very widespread, and would probably not require conservation measures. As outlined above, future research should expand upon our work by sampling more populations and taxa, in order to thoroughly test the circumscriptions of the *A. glandulosa* subspecies. Such work will lead to conservation outcomes that are consistent with the underlying genetics of the plants being conserved, rather than the names applied to them.

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APPENDIX 1

DETAILED COLLECTION INFORMATION FOR SAMPLED LOCALES

For each sampled locale (Table 1), the format is as follows: collector name and number (herbarium of voucher deposition), description of locality, (GPS coordinates), political region (county and state in the case of California; country and state in the case of Mexico).

Arctostaphylos glandulosa subsp. *atumescens*—D. Burge 2006 (SD), 18 Jan 2016, Cerro Buenavista (GPS [NAD84]: 31.6737, -116.6314), Baja California, Mexico.

Arctostaphylos glandulosa subsp. *crassifolia*—D. Burge 1694 (DAV), 18 Mar 2015, Cerro del Coronel (GPS [NAD84]: 32.283, -116.93), Baja California, Mexico. D. Burge 1701 (DAV), 19 Mar 2015, Cañon San Isidro (GPS [NAD84]: 31.292, -116.3434), Baja California, Mexico. D. Burge 1703 (DAV), 20 Mar 2015, Mesa de Descanso (GPS [NAD84]: 32.1718, -116.8898), Baja California, Mexico. D. Burge 1709 (DAV), 21 Mar 2015, Encinitas Community Center (GPS [NAD84]: 33.0444, -117.2669), San Diego Co., CA. D. Burge 1717 (DAV), 3 Apr 2015, Torrey Pines (GPS [NAD84]: 32.9406, -117.2471), San Diego Co., CA. D. Burge 1719 (DAV), 4 Apr 2015, MCAS 1 (GPS [NAD84]: 32.9164, -117.0398), San Diego Co., CA. D. Burge 1720 (DAV), 4 Apr 2015, MCAS 2 (GPS [NAD84]: 32.8897, -117.064), San Diego Co., CA. D. Burge 1722 (DAV), 4 Apr 2015, MCAS 3 (GPS [NAD84]: 32.8787, -117.0659), San Diego Co., CA. D. Burge 1723 (DAV), 4 Apr 2015, MCAS 4 (GPS [NAD84]: 32.8649, -117.069), San Diego Co., CA. D. Burge 1725 (DAV), 4 Apr 2015, MCAS 5 (GPS [NAD84]: 32.8932, -117.0757), San Diego Co., CA. D. Burge 1729 (DAV), 4 Apr 2015, Crest Canyon (GPS [NAD84]: 32.9501, -117.2538), San Diego Co., CA. D. Burge 2071 (SD), 21 Feb 2016, Encinitas (GPS [NAD84]: 33.036, -117.2487), San Diego Co., CA. M. Mulligan 3318 (SD), 12 Feb 2016, MCAS 18 (GPS [NAD84]: 32.8938, -117.0398), San Diego Co., CA. M. Mulligan 3321 (SD), 12 Feb 2016, MCAS 17 (GPS [NAD84]: 32.8915, -117.0516), San Diego Co., CA.

Arctostaphylos glandulosa subsp. *glandulosa*—D. Burge 1523 (DAV), 23 Mar 2014, Cavedale Road (GPS [NAD84]: 38.3624, -122.4719), Sonoma Co., CA. D. Burge 1688 (DAV), 17 Mar 2015, Newhall Pass (GPS [NAD84]: 34.3471, -118.5102), Los Angeles Co., CA. D. Burge 1746 (DAV), 5 Jun 2015, Mount Tamalpais (GPS [NAD84]: 37.911, -122.5775), Marin Co., CA. D. Burge 2033 (DAV), 31 Jan 2016, Bolinas Ridge (GPS [NAD84]: 37.9468, -122.6665), Marin Co., CA. D. Burge 2087 (SD), 22 Feb 2016, Viejas Mountain (GPS [NAD84]: 32.854, -116.7421), San Diego Co., CA. D. Burge 2090 (DAV), 23 Feb 2016, Santa Ana Mountains (GPS [NAD84]: 33.6535, -117.4455), Orange Co., CA.

CLARIFYING THE CONSERVATION STATUS OF NORTHERN CALIFORNIA BLACK WALNUT (*JUGLANS HINDSII*) USING MICROSATELLITE MARKERS

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ABSTRACT

The conservation status of the northern California black walnut (*Juglans hindsii* (Jeps.) Jeps. ex R. E. Sm.) has been a source of considerable confusion and controversy. Although not currently legally protected by either Federal or State Endangered Species Acts, this species is given conservation status by the California Native Plant Society and the California Department of Fish and Wildlife, and some California counties require mitigation for removal of individuals of this species, especially older trees. Despite the current widespread distribution in northern California and southern Oregon of trees that match *J. hindsii* morphologically, there are only three or four sites where the species is known to have occurred prior to extensive settlement of California by Europeans in the mid-19th century. This has led to the suspicion that trees found in other places may not be genetically pure *J. hindsii* but may instead be descendants of lineages that experienced past gene flow from one or more other species. In addition, despite its more distant relationship, the cultivated walnut (*J. regia* L.) readily hybridizes (as the male parent) with *J. hindsii*, producing morphologically identifiable ‘Paradox’ hybrids, which occur spontaneously and are widely planted as rootstocks and street trees. Finally, recent collections of *J. hindsii* from southern California have raised questions about the respective geographic distributions of *J. hindsii* and southern California black walnut (*J. californica* S. Watson). We analyzed genotypes at 10 microsatellite loci for 158 mostly wild *J. hindsii* trees, as well as some orchard waifs, from 10 counties in northern and southern California and one county in southern Oregon, including representatives of putative original native populations. We also sampled several Paradox hybrids, 10–20 standards for each of the five North American black walnut species, and six standards for *J. regia*. Bayesian cluster analyses with the program STRUCTURE revealed that at least 71.5% of the putatively wild *J. hindsii* represent genetically pure members of that species, while the remaining trees show evidence of past hybridizations with one or more of the other North American black walnut species. We found no evidence of introgression of *J. regia* into *J. hindsii*. The results suggest that individual *J. hindsii* trees should not have conservation status.

Key Words: hybridization, introgression, native species, naturalized species, Paradox walnut, protected species, rare species, SSR.

Two species of black walnut (*Juglans* sect. *Rhysocaryon*) are native to California: *J. hindsii* (Jeps.) Jeps. ex R. E. Sm., the northern California black walnut, and *J. californica* S. Watson, the southern California black walnut. Other North American black walnut species, including eastern black walnut (*J. nigra* L.), Arizona walnut (*J. major* [Torrey] A. Heller), and Texas black walnut (*J. microcarpa* Berland.), are occasionally cultivated in

California. Persian (aka English) walnut (*J. regia* L., a member of *Juglans* section *Juglans*) is a major orchard crop in the state and occasionally escapes as a waif but is not considered naturalized (Baldwin et al. 2012). *Juglans hindsii* has been widely planted in California as a street tree and as a rootstock for *J. regia*.

Most species of *Juglans* are interfertile, and the pioneering horticulturist Luther Burbank conducted

crossing experiments and named two hybrids involving California walnuts, 'Royal' (*J. hindsii* × *J. nigra*) and 'Paradox' (*J. hindsii* × *J. regia*) (Burbank 1914; Howard 1945). Burbank first observed hybrids between *J. hindsii* and *J. regia* in 1878 (Howard 1945); he bestowed upon them the name 'Paradox' in 1893, in recognition of their rapid growth and low nut production, factors that have contributed to their popularity as shade trees. He was particularly impressed by the high quality of the wood, considering the primary economic promise of Paradox hybrids to lie in their potential value as timber trees. Subsequently, Paradox was found to be superior in several qualities to *J. hindsii* as a rootstock for *J. regia* (Smith et al. 1912; Catlin 1998), and, while both are still used, Paradox is now the most extensively used rootstock for *J. regia* in California (McGrath and Catlin 1987; Kluepfel et al. 2012). Paradox hybrids occur spontaneously in areas where *J. hindsii* grows in proximity to *J. regia* orchards; they are readily distinguished from both parents by their intermediate leaf morphology and bark color and by their remarkable vigor. Like *J. hindsii*, Paradox hybrids have also been widely planted as street trees. Available evidence indicates that all species and hybrids of *Juglans* are diploids, with $2n = 32$ (Elias 1972).

Although there is no question that *J. hindsii* is native to California, there is considerable confusion and controversy over its indigenous range and conservation status. In addition to extensive plantings in urban areas and orchards, trees that match *J. hindsii* morphologically occur widely in unmanaged habitats, especially riparian areas, in central and northern California, where they are generally considered "naturalized" (Griffin and Critchfield 1972). Nonetheless, the species has been given rarity status by the California Native Plant Society (CNPS 2017) and California Department of Fish and Wildlife (CNDDB 2017), and, although it is not currently officially listed by either the State or Federal Endangered Species Acts (ESA), some California counties require mitigation for removal of individuals this species, especially older trees. Furthermore, under the California Environmental Quality Act, species that are not currently officially listed by either ESA still require actions during pre-project review, including surveys, disclosure of what is present and its significance, and proposed mitigation for any significant losses proposed during the project implementation. The designation of conservation status for *J. hindsii* rests on the fact that, despite its current widespread distribution, there are only three (Smith 1909; Smith et al. 1912), or possibly four (Jepson 1917) sites, in Contra Costa, Sacramento, and Napa Counties, where the species was confirmed to have occurred prior to extensive settlement of California by Europeans in the mid-19th century. These have generally been accepted as the only sites where the species should be considered indigenous, rather than having been planted intentionally or escaped from

intentional plantings and naturalized (Kirk 2003). According to CNPS (2017), only one of these sites is considered viable and the species is threatened by changes in land use and by hybridization with *J. regia*. These assertions have led to widespread concern among individuals and agencies in northern California that trees occurring in other places may not be genetically pure *J. hindsii*, but instead be descended from lineages that experienced past gene flow from one or more other *Juglans* species. By contrast, Callahan (2008) considered it likely that *J. hindsii* occurs at multiple additional locations, including sites in southern Oregon, beyond those documented by Smith et al. (1912) and Jepson (1917). Results from analyses of restriction fragment length polymorphisms (RFLPs; Fjellstrom and Parfitt 1994) revealed reduced genetic diversity in *J. hindsii* compared to other *Juglans* species, consistent with a past genetic bottleneck as would be expected if extant members of the species were derived from a relatively small number of ancestral populations.

The difficulty of pinpointing the original sites where *J. hindsii* occurred prior to the mid-19th century is compounded by the fact that extensive movement and planting of the species and various hybrids by people as well as changes in land use have resulted in the occurrence of trees in wild-looking places which in fact represent rootstocks from old orchards or other past intentional plantings. Thus, extant populations almost certainly represent a mixture of naturally occurring and intentionally planted stands, as well as of spontaneous and intentionally produced hybrids.

To date, two published studies have used molecular markers to address the question of genetic purity of *J. hindsii* trees from locations other than one of the putative native sites. The first (Potter et al. 2002) focused on investigating the genealogies of Paradox sources, the individual mother trees from which Paradox rootstock seedlings are obtained, using data from single nucleotide polymorphisms (SNPs) derived from nucleotide sequences from the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (biparentally inherited) and three non-coding regions of plastid DNA (maternally inherited). Of 27 Paradox sources tested, 18 were found to be pure *J. hindsii* based on the markers used, while the remainder showed contributions from *J. nigra*, *J. major*, or *J. californica* as maternal and/or paternal parents, as well as *J. hindsii*, in their backgrounds. Similar results (i.e., that most *J. hindsii* are genetically pure, but some individuals show evidence of past gene flow from other species) have been observed in testing of additional trees, using the same markers, over the past decade (Potter, unpublished data).

The second published study (Kirk 2003) examined variation in ITS sequences of eight putatively naturalized (escapes from intentional plantings) populations of *J. hindsii* in the Sacramento Valley, as well as two of the putative native populations, and

representatives of the other species present in California. The results suggested the possibility of past gene flow from *J. major* into both native and naturalized populations of *J. hindsii*.

There are several limitations of the aforementioned studies. First, only one nuclear genomic region was sampled and because concerted evolution tends to homogenize ribosomal DNA, including ITS (Wendel et al. 1995), polymorphisms present in first-generation hybrids could be lost in later generations, precluding the possibility of detecting gene flow from other species as ancestors more remote than parents or grandparents. Second, not surprisingly, low levels of DNA sequence variability were observed among these closely related species, so conclusions were based on just six SNPs in the ITS and ten in the plastid DNA sequences. Finally, only a limited number of representatives of other species, especially *J. major* (five and three individuals, respectively, in Potter et al. 2002 and Kirk 2003) were included. This is not meant to suggest that these past studies and the markers they employed did not provide valuable information, but their value would be significantly enhanced by expanded sampling and additional evidence from other markers. In this regard, the microsatellite markers developed for *J. nigra* (Woeste et al. 2002; Robichaud et al. 2006) and successfully applied to studies of genetic diversity and cultivar identification in *J. regia* (Dangl et al. 2005) are particularly promising.

Two additional issues require further investigation. The first is that, although gene flow from *J. regia* to *J. hindsii* is considered unlikely due to male sterility and very low fruit set by Paradox hybrids, anecdotal reports that some Paradox individuals do produce large numbers of viable seeds (e.g., Robinson, Sierra Gold Nurseries, personal communication) cast some doubt on the certainty of this conclusion. Thorough genetic testing is needed to assess definitively whether gene flow from cultivated to wild walnuts is a concern. The second issue requiring clarification is the presence of spontaneous populations of *J. hindsii* in southern California, recorded from herbarium specimens collected in five counties (Los Angeles, Orange, Riverside, San Bernardino, and San Diego) over the past five years (Sanders, University of California Riverside Herbarium, personal communication; Consortium of California Herbaria 2017). Genetic testing is important for clarifying the identification and elucidating the origins of these populations.

In order to address persistent questions about the genetic identities of putatively wild walnut trees in California and southern Oregon, and to test the hypothesis that most of them are pure *J. hindsii*, we analyzed genotypes at 10 microsatellite loci for 158 putatively wild trees of *J. hindsii* from 10 counties in northern and southern California, and from one county in southern Oregon including representatives of putative original indigenous populations. We also

included several Paradox hybrids, 10-20 standards for each of the five North American black walnut species, and six standards for *J. regia*.

MATERIALS AND METHODS

Sampling

A total of 249 trees were sampled for this study, of which 167 were new field collections (Fig. 1, Supplemental Table S1) from localities in Jackson County, southern Oregon (13 trees) and 10 California counties (Alameda [n = 1]; Contra Costa [n = 64]; El Dorado [n = 5]; Los Angeles [n = 4]; Napa [n = 66]; Orange [n = 3]; San Bernardino [n = 2]; San Diego [n = 5]; Santa Clara [n = 1]; Sonoma [n = 3]). Most (158) of these trees were identified as *J. hindsii* at the time of collection based on morphology. Fifteen of the trees were collected from two of the putative original native populations (10 from Las Trampas Creek in Contra Costa County and 5 from Circle Oaks Drive at the Capell Creek/Wooden Valley site in Napa County; CNDDDB 2017). We also included seven trees identified as Paradox hybrids and two samples from Los Angeles County identified as *J. californica*. The majority of these samples (134) were collected by Heath Bartosh; additional collections were contributed by Richard Riefner (14 collections from southern California), Frank Callahan (10 collections from Oregon), Dan Potter (five collections from El Dorado County), Brian Peterson (three collections from Oregon), and Chris Jannusch (one collection from Santa Clara County). Voucher specimens for samples collected by Bartosh, Riefner, and Potter were deposited in the herbarium of the Center for Plant Diversity at UC Davis (DAV).

In addition, we included 10-20 standards for each of the five North American black walnut species plus six standards for *J. regia* (Table S2). These species standards comprised 66 individuals from the collection of the USDA National Clonal Germplasm Repository (further details about these accessions are available at <https://npgsweb.ars-grin.gov/gringlobal/search.aspx?>), identified as *J. hindsii* (n = 10), *J. californica* (n = 16), *J. major* (n = 14), *J. microcarpa* (n = 16), *J. nigra* (n = 4), and *J. regia* (n = 6), as well as 16 accessions of *J. nigra* from the collections of the Hardwood Tree Improvement & Regeneration Center (HTIRC), USDA-Forest Service, Northern Research Station, associated with Purdue University, kindly provided by Keith Woeste and James McKenna. The majority of these individuals originated from seeds collected from wild trees. As described below, microsatellite marker genotypes suggested that 11 of these 82 species standards did not represent genetically pure members of the species to which they were assigned; those individuals were excluded from final analyses. Thus, for our final analyses, we

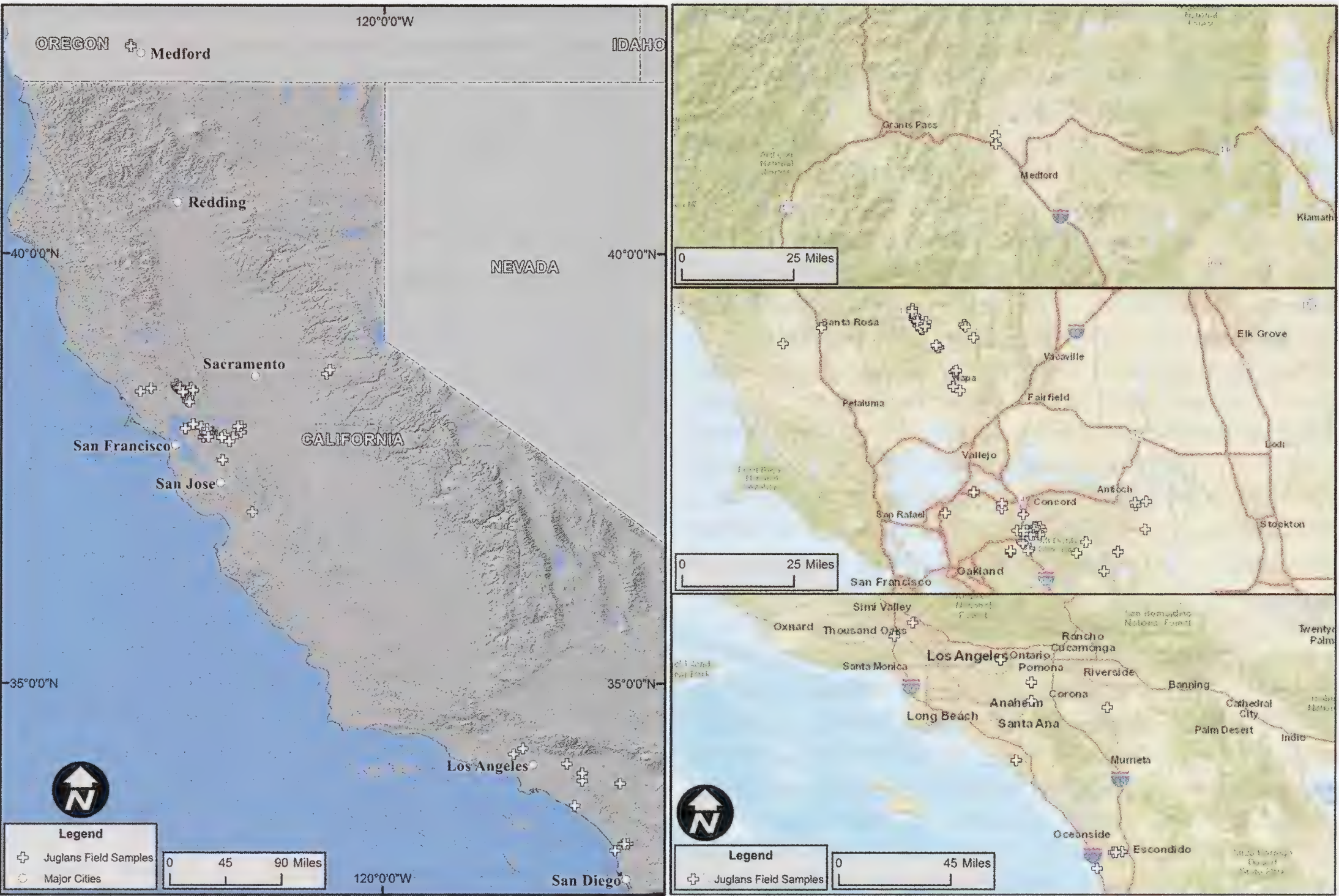


FIG. 1. Overview of all collection localities (left) and close-ups (right) of collection localities in southern Oregon (top), the San Francisco Bay Area (middle), and southern California (bottom) for the 167 field-sampled trees included in this study.

analyzed the genotypes of 238 trees (71 species standards + 167 field collected trees).

Genotyping

Collected samples consisted of young, fresh, green leaves that were dried using chemical desiccants (Bautista et al. 2008). Whole genomic DNA was extracted by a modified CTAB protocol adapted from Doyle (1991). Samples were analyzed at 12

microsatellite loci. These loci were originally developed from *J. nigra*, as described in Woeste et al. (2002); primer sequences are found in Dangl et al. (2005). Two loci (WGA202 and WGS349) produced multi-locus genotypes across several species and were excluded from final analyses. The 10 loci used in the final analyses are listed in Table 1. A standard set of PCR conditions was used for all samples (Dangl et al. 2005). The fragment separation and sizing were performed as previously described for *Vitis* species

TABLE 1. STATISTICS (NUMBER AND PERCENTAGE OF MISSING DATA POINTS, NUMBER OF ALLELES (N_a), NUMBER OF EFFECTIVE ALLELES (N_e), INFORMATION INDEX (I), OBSERVED HETEROZYGOSITY H_o), EXPECTED HETEROZYGOSITY (H_e), UNBIASED EXPECTED HETEROZYGOSITY (uH_e), AND FIXATION INDEX (F)) OF SSR LOCI AMPLIFIED FOR THIS PROJECT BASED ON THE FINAL 238 SAMPLES ANALYZED. Designations for SSR primer pairs follow Dangl et al. (2005).

Locus	Missing #	Missing %	N_a	N_e	I	H_o	H_e	uH_e	F
WGA 001	150	63.0	18	10.149	2.483	0.455	0.901	0.907	0.496
WGA 276	1	0.4	24	4.682	2.107	0.570	0.786	0.788	0.276
WGA 376	2	0.8	12	5.492	2.024	0.631	0.818	0.820	0.228
WGA 009	15	6.3	16	5.411	2.061	0.704	0.815	0.817	0.136
WGA 118	5	2.1	27	3.876	2.019	0.536	0.742	0.744	0.277
WGA 089	0	0.0	28	2.435	1.640	0.424	0.589	0.591	0.280
WGA 331	1	0.4	19	3.124	1.713	0.494	0.680	0.681	0.274
WGA 321	136	0.6	20	11.098	2.610	0.480	0.910	0.914	0.472
WGA 332	1	0.4	21	3.984	1.999	0.574	0.749	0.751	0.234
WGA 069	0	0.0	11	1.896	1.151	0.248	0.472	0.473	0.475
Mean	31.1	7.4%	19.6	5.215	1.981	0.512	0.746	0.749	0.315

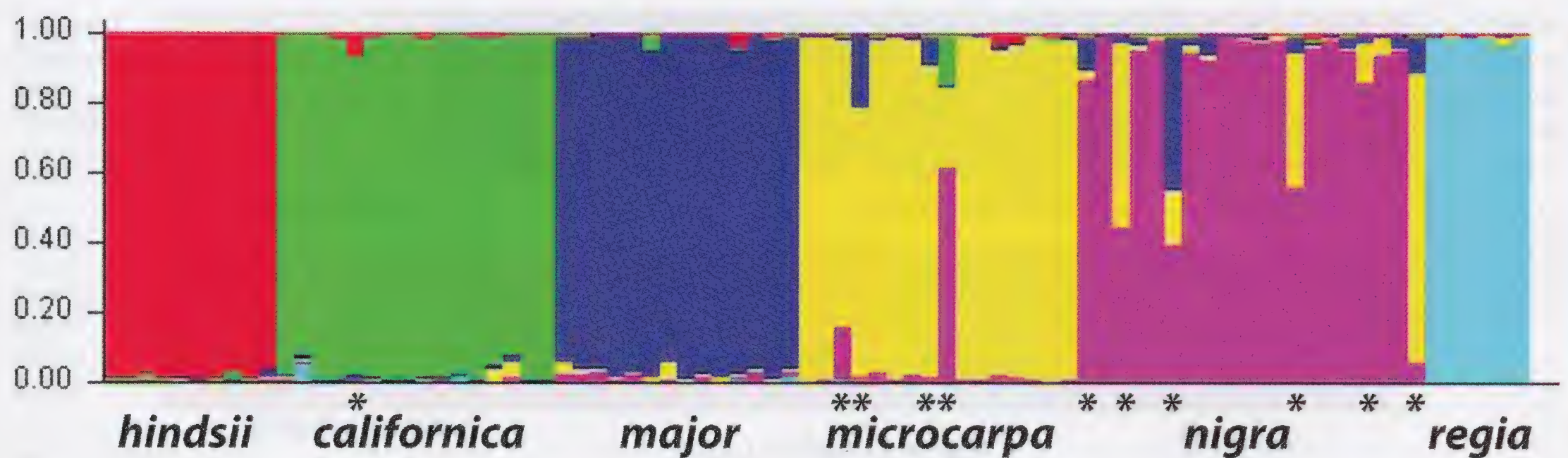


FIG. 2. Sample result of STRUCTURE analysis ($K = 6$) of the 82 species standards included in this study. Individuals marked with asterisks were excluded from final analyses (see Table S2).

(Dangl et al. 2015). Genotypes were assigned using the software package STRand (Toonen and Hughes 2001; <http://www.vgl.ucdavis.edu/STRand>).

Data Analyses

In order to assess levels of genetic diversity in *J. hindsii* compared to other walnut species, basic statistics (including numbers of alleles and observed and expected heterozygosities for each locus and each species) were calculated using GenAlEx (Peakall and Smouse 2012). To investigate the number of genetic clusters among the samples based on our microsatellite marker data, Principal Coordinate Analyses (PCoA) of the data were conducted using GenAlEx and Bayesian clustering analyses were implemented in STRUCTURE (Pritchard et al. 2000). For all Bayesian clustering analyses, the admixture ancestry model, in which each individual can be assigned to more than one cluster, was used, allowing identification of hybrids. Further details about these analyses are provided in the Results section.

RESULTS

Eight of the 10 primer pairs produced amplification bands in the all or nearly all of the 238 individuals (Table 1). Specifically, two (WGA 089, WGA 069) succeeded in all individuals, three (WGA 276, WGA 331, and WGA 332) each failed in one individual, one (WGA 376) failed in two individuals, one (WGA 118) failed in five individuals, and one (WGA 009) failed in 15 individuals. For those eight primers, the individuals for which no products were obtained were treated as missing data for all analyses. Two primer pairs failed in a substantially larger number of individuals. Primer pair WGA 001 failed in 150 individuals (63.0%), including nine species standards for *J. hindsii* and 10 for *J. californica* as well as 131 of the field collections, while primer pair WGA 321 failed in 136 individuals (59.5%), including all 10 species standards for *J. hindsii*, one for *J. californica*, and 125 of the field collections. For GenAlEx analyses, all of these cases were coded as missing data. For the 10 microsatellite

loci across the 238 individuals included in the final analyses, the numbers of observed and effective alleles, observed heterozygosity, and expected heterozygosity ranged from 11 to 28, 1.9 to 11.1, 0.25 to 0.70, and 0.47 to 0.91, respectively (Table 1).

Because the large number of failed amplifications with limited taxonomic distribution suggested the possibility of presence of a null allele in the two native California species at the microsatellite loci amplified by primer pairs WGA 001 and WGA 321, an approach that allows for this possibility was used for those loci in the Bayesian clustering analyses. Specifically, presence of a possible recessive allele, i.e., a change in sequence such that no PCR amplification product is generated, was indicated for each of the two loci, and individuals for which amplification failed were coded as homozygotes for that allele, following the instructions in the STRUCTURE manual (Pritchard et al. 2000).

The results of PCoA were generally consistent with those from Bayesian clustering analyses; only the latter will be presented here. Bayesian clustering analyses were conducted in three phases. The goal of the first phase was to ensure the reliability of our standards for *J. hindsii* and each of the other potential parental lineages. Thus, only the genotypes of the 82 individuals initially designated as species standards were included. The number of genetic clusters (K) was set at 6, corresponding to the six species, with the genetic admixture ancestry model in effect, and no *a priori* information about the expected assignment of each individual was provided. The clustering procedure assigns each individual to one or more of the six clusters. We calculated the average assignment coefficient (q) of each individual to each of the six species across 25 STRUCTURE runs. Based on the findings and recommendations of previous studies (Vähä and Primmer 2006; Lepais et al. 2009), we set the threshold for designating an individual as a genetically pure member of a species at $q \geq 0.90$. Using this criterion, our results showed that 11 of the species standards (one individual of *J. californica*, four of *J. microcarpa*, and six of *J. nigra*) were hybrids with one or more other species (Fig. 2, Table S2). These samples were excluded from further

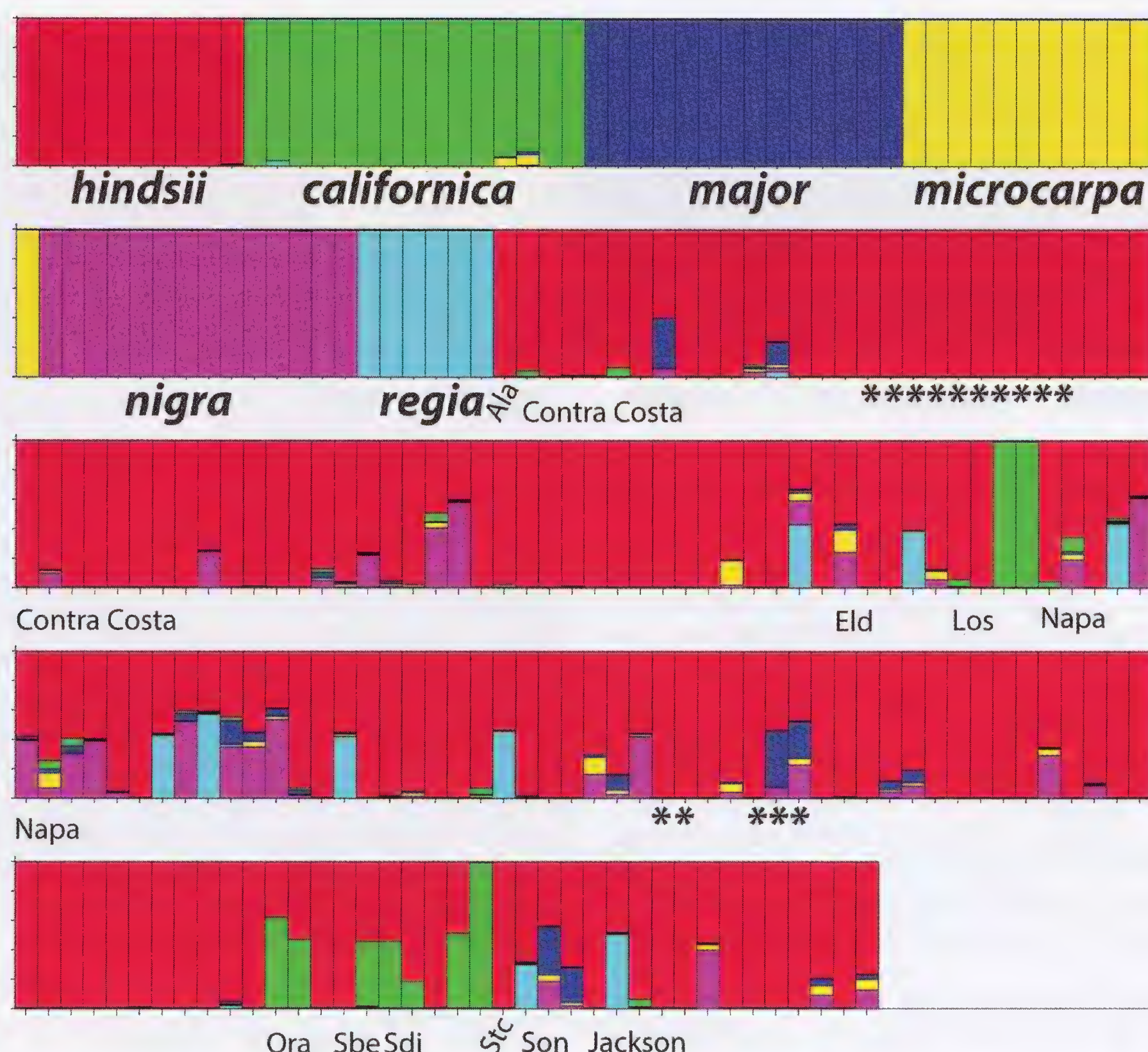


FIG. 3. Sample result of STRUCTURE analysis ($K = 6$) for the 71 final species standards (Table S2) plus the 167 field-sampled trees, presented in the same order as in Table S1, included in this study. County name abbreviations: Ala = Alameda; Eld = El Dorado; Los = Los Angeles; Ora = Orange; Sbe = San Bernardino; Sdi = San Diego; Stc = Santa Clara; Son = Sonoma. Individuals marked with asterisks were collected from putative original populations of *J. hindsii* in Contra Costa and Napa Counties (see Table S1).

analyses; thus the final number of species standards was 71, comprised of 10 *J. hindsii*, 15 *J. californica*, 14 *J. major*, 12 *J. microcarpa*, 14 *J. nigra*, and six *J. regia*.

The goal of the second phase of the clustering analyses was to check the species assignments of the 167 field-sampled trees (158 collected as pure *J. hindsii*, seven as Paradox hybrids, and two as pure *J. californica*). Thus, in this phase, the genotypes of the 167 field collections were added to the 71 species standards. The number of clusters was set at 6, each of the 71 species standards was flagged as belonging to its respective cluster, but no *a priori* information about the expected assignment of any of the 167 field collections was provided. We calculated the average assignment of each of the 167 trees to each of the six species across 25 STRUCTURE runs (example in Fig. 3), and the results were used to designate each individual as either a pure member of one of the species or a hybrid with two or more species in its ancestry.

Genealogical histories were inferred based on the relative percentages of assignment to each species,

with inferences of hybridization limited to the three previous generations. Individuals that were assigned at $q < 0.90$ to *J. hindsii* were considered putative hybrids, while $q = 0.10$ was in most cases set as the minimum assignment value of an individual to a species required for that species to be considered an ancestor of that individual. In eight cases, individuals assigned to *J. hindsii* at $q < 0.90$ but did not assign to any other species at $q \geq 0.10$. For each of those individuals, the species with the next-highest value of q was designated as a potential great-grandparent of the individual, but with a question mark to due to our lack of confidence in these inferences (Tables 2, S1).

Of the 158 trees identified in the field as *J. hindsii* based on morphology, 113 (71.5%) were found to be pure members of that species based on their microsatellite marker genotypes, while the seven trees identified as Paradox hybrids and two identified as *J. californica* based on morphology were confirmed as such (Table 2). The remaining 45 putative *J. hindsii* were found to be F1 (*J. hindsii* \times *J. californica*, *J. hindsii* \times *J. major*, or *J. hindsii* \times *J.*

TABLE 2. SUMMARY OF GENEALOGICAL HISTORIES OF THE 167 FIELD-SAMPLED TREES INCLUDED IN THIS STUDY, BASED ON THE RESULTS OF STRUCTURE ANALYSES. Species name abbreviations: cal = *J. californica*; hin = *J. hindsii*; maj = *J. major*; mic = *J. microcarpa*; nig = *J. nigra*; reg = *J. regia*. County name abbreviations: Ala = Alameda; Eld = El Dorado; Los = Los Angeles; Ora = Orange; Sbe = San Bernardino; Sdi = San Diego; Stc = Santa Clara; Son = Sonoma.

Inferred ID	Total	County										
		Ala	Con	Eld	Los	Nap	Ora	Sbe	Sdi	Stc	Son	Jac
hin	113	1	54	2	2	41	1	1	1	1		9
hin × (hin × (hin × maj?))	2					2						
hin × (hin × (hin × mic?))	1					1						
hin × (hin × (hin × nig?))	5		1	1		2						1
hin × (hin × (hin × nig))	1		1									
hin × (hin × cal)	1								1			
hin × (hin × maj)	3		2								1	
hin × (hin × mic)	2		1			1						
hin × (hin × nig)	7		2			4						1
hin × (hin × reg)	1										1	
hin × (hin × (nig × mic))	1					1						
hin × (maj × nig)	2					2						
hin × (nig × mic)	1			1								
hin × (maj × (maj × nig))	1										1	
hin × cal	5						2	1	2			
hin × maj	1					1						
hin × nig	9		2			6						1
Paradox	7			1		5						1
(hin × (hin × nig)) × reg	1		1									
cal	3				2				1			
Total	167	1	64	5	4	66	3	2	5	1	3	13

nigra) or more complex hybrids. Among the trees collected from the putative original locations for the species, all 10 from the Las Trampas site in Contra Costa County and three from the Circle Oaks Drive site in Napa County were identified as pure *J. hindsii*, while two from the latter site were identified as hybrids involving *J. major* and *J. nigra* (Fig. 3, Table S1). Of 12 trees from southern California identified as *J. hindsii* at the time of collection based on morphology, five were confirmed as such, five were found to be F1 hybrids between *J. hindsii* and *J. californica*, one was determined as *J. hindsii* × (*J. hindsii* × *J. californica*), and one was assigned completely to *J. californica* by our analyses.

In the third phase of Bayesian clustering analysis, only the 113 individuals identified as pure *J. hindsii* in the second phase were included in order to test for evidence of genetic structuring within the species. For all four values of K tested (2, 3, 4, and 5), contributions from all gene pools were detected in all individuals, albeit to varying degrees of assignment (Fig. 4), suggesting no significant genetic structuring within the species based on the samples included here.

Analyses of average heterozygosity, F-statistics, and polymorphism across the 10 loci for each species, with putative hybrids excluded, revealed that *J. hindsii* had the lowest genetic diversity (as measured by numbers of effective alleles, information index,

and observed and expected heterozygosity) of the six species sampled (Table 3).

DISCUSSION

Bayesian cluster analyses with the program STRUCTURE revealed that at least 71.5% of the putatively wild *J. hindsii* represent genetically pure members of that species; the proportion could be as high as 76.6% since the contributions of other species as great-grandparents were considered questionable in eight cases. The remaining trees show evidence of past hybridizations with one or more of the other North American black walnut species. Only one sample (HB 1299) showed evidence of introgression of *J. regia* into *J. hindsii* beyond first-generation Paradox hybrids. This was a tree from Luther Burbank’s home in Sonoma County whose ancestry was inferred as *J. hindsii* × (*J. hindsii* × *J. regia*) and may represent the result of an intentional cross. The tree was noted as a potential hybrid at the time of collection. These results suggest that, as expected, widespread planting of *J. regia* is not a significant threat to genetic purity of *J. hindsii*.

Our sampling was not designed to test any specific hypotheses about the precise geographic origin of *J. hindsii* as a species or the histories of establishment of extant populations (whether natural or anthropogenic) and, given the very limited number of individuals present at the putative original sites for the species (CNDDDB 2017), it would be difficult, if

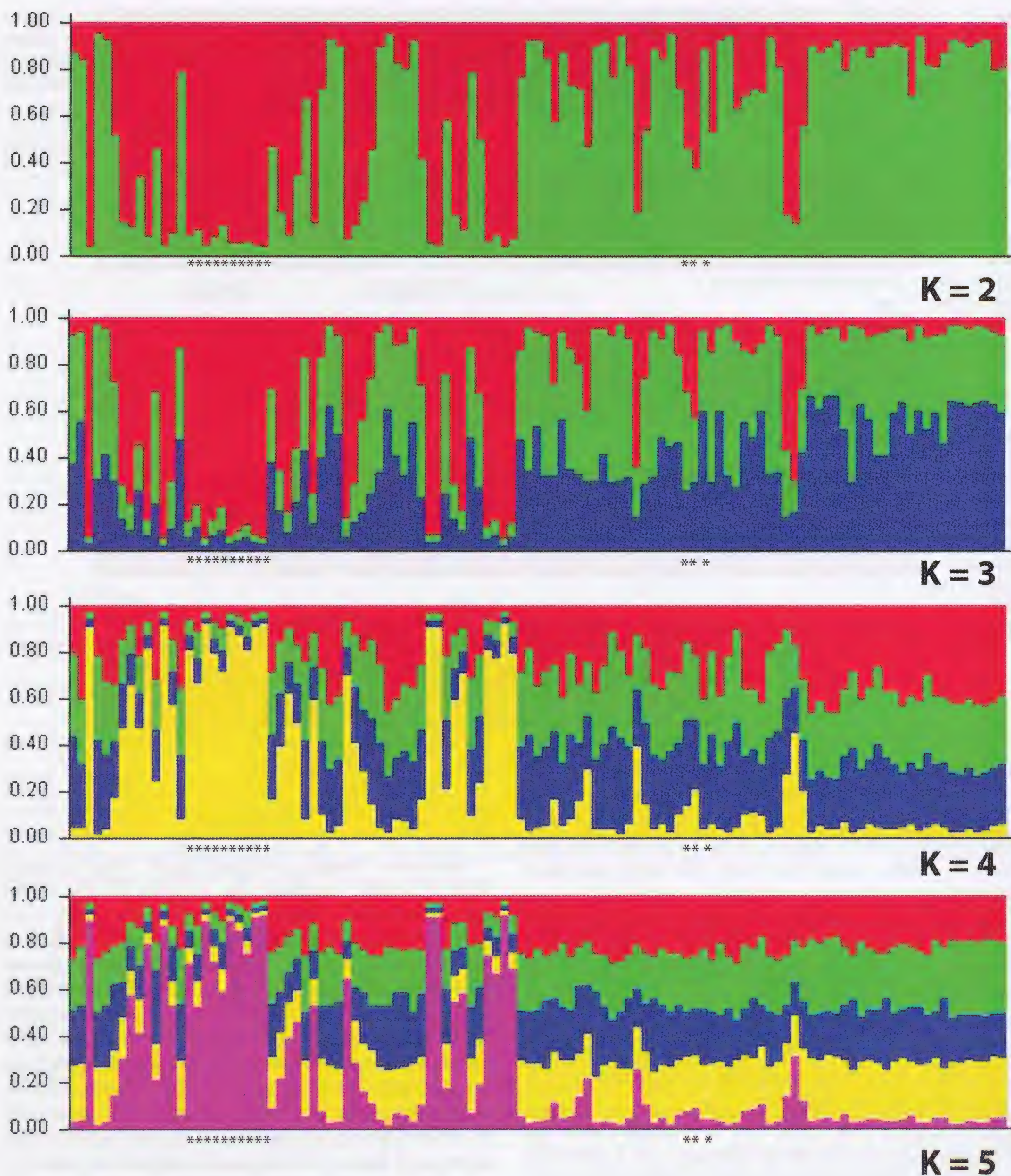


FIG. 4. Sample results of STRUCTURE analyses ($K = 2, 3, 4$, and 5) for the 113 field-sampled trees included in this study that were inferred to be pure *J. hindsii*. Individuals marked with asterisks were collected from putative original populations of *J. hindsii* in Contra Costa and Napa Counties (see Table S1).

not impossible, to conduct a study that would rigorously test any such hypotheses. Not surprisingly, the results of our STRUCTURE analyses including only individuals identified as pure *J. hindsii* (Fig. 4) suggest that, on one hand, the greatest genetic diversity was captured within the two most extensively sampled counties, Contra Costa and Napa, while, on the other hand, the full range of

diversity was not captured among the very limited number of trees collected at the two putative original sites (Fig. 4). Nonetheless, we found no evidence of geographically based genetic structuring within *J. hindsii* across the areas sampled here. Of the 14 trees sampled from southern California, the six that were identified as pure *J. hindsii* are almost certainly the result of human introductions, as there is no

TABLE 3. HETEROZYGOSITY, F-STATISTICS, AND POLYMORPHISM BY SPECIES. Putative hybrids based on prior STRUCTURE analyses (Tables 2 and S1) were excluded from this analysis. Values reported are means and standard errors (SE) across 10 microsatellite loci (Table 3). n = sample size (in some cases less than number of individuals sampled due to missing data at some loci), N_a = number of alleles; N_e = number of effective alleles; I = information index, H_o = observed heterozygosity, H_e = expected heterozygosity, uH_e = unbiased expected heterozygosity, and F = fixation index.

Species	n	N _a	N _e	I	H _o	H _e	uH _e	F
<i>hindsii</i>								
Mean	94.100	3.700	1.784	0.628	0.281	0.342	0.344	0.159
SE	15.518	0.716	0.232	0.160	0.073	0.086	0.086	0.035
<i>californica</i>								
Mean	16.200	5.700	3.254	1.269	0.562	0.612	0.630	0.101
SE	1.200	0.790	0.451	0.180	0.093	0.076	0.078	0.078
<i>major</i>								
Mean	13.800	8.900	5.650	1.869	0.707	0.801	0.831	0.120
SE	0.200	1.100	0.632	0.124	0.053	0.024	0.025	0.060
<i>microcarpa</i>								
Mean	11.800	7.300	5.090	1.602	0.782	0.698	0.729	-0.125
SE	0.133	1.096	0.967	0.220	0.096	0.084	0.087	0.048
<i>nigra</i>								
Mean	13.600	7.500	4.608	1.676	0.736	0.755	0.784	0.019
SE	0.221	0.563	0.444	0.099	0.038	0.035	0.036	0.039
<i>regia</i>								
Mean	6.000	3.700	2.803	1.111	0.750	0.617	0.673	-0.231
SE	0.000	0.300	0.258	0.083	0.057	0.034	0.037	0.087

historical evidence to suggest that the species is native in those areas, while three trees were in fact *J. californica* and five were hybrids between the two species.

Our finding of lower genetic diversity in *J. hindsii* compared to other species (Table 3), similar to results reported by Fjellstrom and Parfitt (1994) based on RFLPs, is consistent with a past genetic bottleneck as would be expected if extant populations were derived from a relatively small number of ancestral populations. Nonetheless, the widespread occurrence of genetically pure *J. hindsii* suggests that the reduced genetic diversity has not, to date, impeded the persistence and spread of this species and that the CNPS (2017) rare plant rank of 1B.1 (rare, threatened, or endangered in California and elsewhere; seriously threatened in California) is not warranted.

On the other hand, *J. californica*, which showed higher levels of genetic diversity among our samples consisting primarily of germplasm repository accessions, is ranked 4.2 (watch list: plants of limited distribution; moderately threatened in California) by CNPS (2017), but may in fact be more seriously threatened due to extensive urbanization in southern California (Holstein 1984). A thorough study of the conservation status of *J. californica* is needed.

In addition to their implications for conservation and taxonomy, our results confirm the utility of microsatellite loci originally developed from *J. nigra* (Woeste et al. 2002) in other walnut species, as previously shown by Dangl et al. (2005) in *J. regia*; they also demonstrate the importance of careful inspection and interpretation of data when transporting molecular markers across species. Two of the 12 loci we tried to use initially produced multi-locus

genotypes across several species and were excluded from final analyses. Among the remaining 10 loci, two primer pairs each failed in more than 50% of the individuals of *J. hindsii* and *J. californica*. Because of the restricted and concentrated taxonomic distribution of these failed reactions, we are confident in our interpretation that they are due to the presence of a null allele in the two native California species at the loci amplified by primer pairs WGA 001 and WGA 321.

In summary, our results show that genetically pure representatives of *J. hindsii* are common throughout the areas in California and southern Oregon sampled here. The limited, but appreciable, levels of hybridization and introgression from other native North American *Juglans* species probably resulted from occasional past introductions of those species as street trees or rootstocks. There is no evidence, however, of significant introgression from the widely introduced *J. regia*. Taken together, our results indicate that individual *J. hindsii* trees should not be considered a rare or imperiled species as currently treated. This study therefore effectively settles a long-held conservation concern or point of confusion about northern California black walnut trees and their conservation status.

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A NEW TAXONOMY FOR *TRIFOLIUM VARIEGATUM* AND ITS RELATIVES

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ABSTRACT

Two related species of *Trifolium* L. endemic to western North America, *T. variegatum* Nutt. s. l. and *T. polyodon* Greene, are studied. Multivariate analyses and morphological differences suggest that three species should be recognized instead of two. *Trifolium variegatum* is divided into two species: *T. appendiculatum* Lojac. and *T. variegatum*. *Trifolium appendiculatum* (including *T. rostratum* Greene) is a large-flowered species endemic to California and southern Oregon. *Trifolium variegatum* is found throughout western North America and remains the most diverse, but is now circumscribed by a consistent floral morphology. *Trifolium polyodon* is restricted to the Monterey peninsula and has distinctive calyx lobe teeth; its circumscription remains unchanged.

Key Words: Fabaceae, section *Involucrarium*, *Trifolium appendiculatum*, *Trifolium polyodon*, *Trifolium variegatum*.

The genus *Trifolium* L. (the clovers, Fabaceae) are herbaceous plants with often trifoliolate leaves, papilionaceous flowers contained in racemose-umbellate heads to head-like inflorescences, diadelphous stamens, and persistent corollas. This genus has approximately 240 species worldwide (Zohary and Heller 1984). Its centers of diversity are the Mediterranean region and the parts of North and South America with Mediterranean-type climates. Several species, such as *Trifolium repens* L. and *T. pratense* L., are cultivated as forage crops (Zohary and Heller 1984), and thereby have become introduced and naturalized worldwide. A phylogenetic study of the genus based on nuclear ribosomal DNA ITS and chloroplast *trnL* intron sequences by Ellison et al. (2006) confirmed its monophyly.

Zohary and Heller (1984) recognized two lineages of New World clovers: species with a head subtended by an involucre of fused bracts, of which there are approximately 25, were placed in sect. *Involucrarium* Hooker; the remainder of New World clovers, those lacking the involucre, were placed in sect. *Lotoidea* Crantz. Ellison et al. (2006), using molecular data, placed all New World clovers into a more broadly circumscribed section *Involucrarium*. Interestingly, *Involucrarium sensu* Zohary and Heller, with the exception of one species, is contained in two clades emerging from a species-rich polytomy (Ellison et al. 2006). Regardless of whether the involucre is phylogenetically useful or not, it remains a useful field trait. Within *Involucrarium s.s.*, taxonomic importance has been placed on the appearance of the calyx, the involucre bracts, and stipules. Gross aspects of the morphology, such as leaflet shape, can often be misleading. The group consists of both annuals and perennials. Species can be widespread or local endemics. A small subset of these involucre clovers is separated from their counterparts by having narrow

calyx lobes uniformly longer than the calyx tube, a glabrous epidermis, and wheel-like involucre bracts. This includes the commonly-recognized species *T. wormskioldii* Lehm., *T. buckwestiorum* Isely, *T. polyodon* Greene, and *T. variegatum* Nutt. (Vincent and Isely 2012). These species form a clade in the Ellison et al. (2006) phylogeny. *Trifolium wormskioldii* is a perennial species, whereas the remainder of the species are annuals. *Trifolium buckwestiorum* is a rare species endemic to California and is distinguished by its cleistogamous flowers. Likewise, *T. polyodon* is only found in Monterey, CA and is separated from its close relative, *T. variegatum s.l.*, by having 8–14 calyx lobe teeth per calyx. *Trifolium variegatum* typically lacks calyx lobe teeth, but when they are present, have 1–8 calyx lobe teeth per calyx. It has, however, on occasion been lumped with *T. variegatum* (Isely 1993) and with *T. tridentatum* Lindl. (Jepson 1936). *Trifolium variegatum s.l.* is found throughout western North America, ranging from British Columbia in Canada south through the western U.S. (Arizona, California, Idaho, Montana, Nevada, Oregon, Utah, Washington, and Wyoming) and into Baja California, Mexico. It can grow from sea level up to 3,000 m in elevation, in habitats as diverse as vernal pools, chaparral, woodlands, and grasslands, as long as the area is locally moist. *Trifolium variegatum s.l.* and *T. polyodon* form the subject of the present study.

The taxonomy of *T. variegatum s.l.* and *T. polyodon* has been problematic. The group is circumscribed by an annual life cycle, glabrous epidermis, wheel-like involucre bracts, and uniformly-long calyx lobes. There is considerable phenotypic diversity. Various morphologies have been identified as worthy of specific recognition, with a resulting 16 epithets being proposed over the past 125 years. Proposed taxa were based on traits such as the number of flowers per inflorescence, the size of the vegetative structures, and

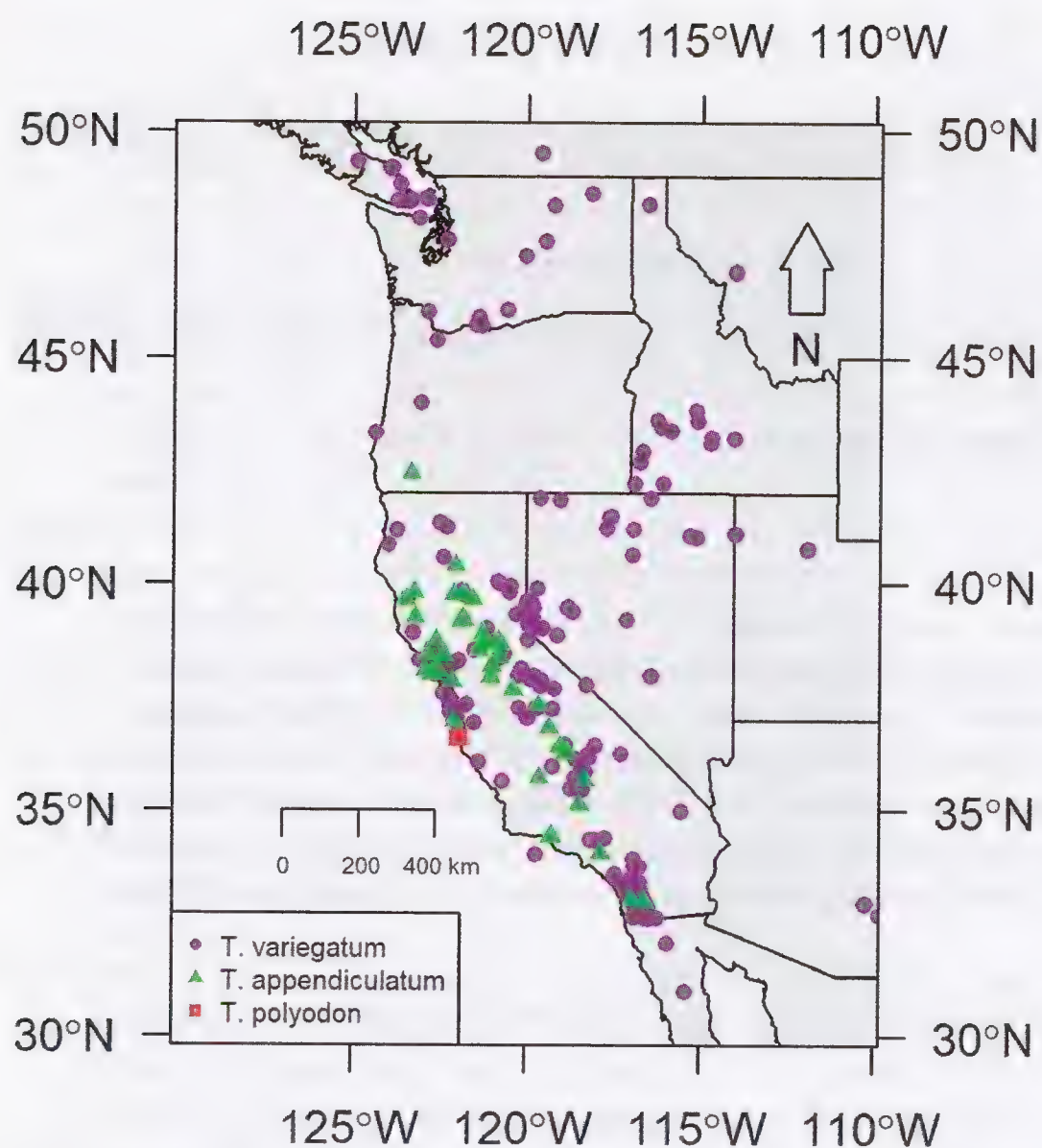


FIG. 1. Map of *T. variegatum* complex. Collections are mapped according to information derived from label data. Specimens are labeled according to taxa recognized in this study. Mapped specimens include those used in statistical analyses in addition to those not included.

peculiar floral morphologies. Recent publications covering this group include Zohary and Heller's (1984) monograph and the treatment of Vincent and Isely (2012) for Californian taxa. In both of these publications, *T. polyodon* is recognized as a distinct species. Each treatment divides *T. variegatum* into varieties. Zohary and Heller (1984) recognized two: *T. variegatum* var. *variegatum* and *T. variegatum* var. *rostratum* (Greene) J. Martin emend. D. Heller (nom. inval.; art. 30.8). These varieties are distinguished according to the absence or presence of a small beak on the keel petals of the corolla. Vincent and Isely (2012) recognized three varieties of *T. variegatum*: *T. variegatum* var. *geminiflorum* (Greene) Vincent, *T. variegatum* var. *major* Lojac., and *T. variegatum* Nutt. var. *variegatum*. The first variety represents a slighter and fewer-flowered form, the second variety has a more robust growth form, and the third variety shows the typical phenotype. Earlier treatments, such as the *Manual of Flowering Plants of California* (Jepson 1925) and *A California Flora* (Munz and Keck 1959), offer similar taxonomies, with the addition of a segregate: *T. appendiculatum* Lojac. This species roughly correlates to the previously mentioned *T. variegatum* var. *rostratum* (Zohary and Heller 1984) and *T. variegatum* var. *major* (Vincent and Isely 2012).

In order to clarify the taxonomy of this group, we assembled a number of herbarium specimens and investigated the breadth of its morphological and geographical diversity. This investigation of *T. variegatum* and its allies began with five groups (Foster 2015); their status as species was tested. After a multivariate analysis, three groups meriting recog-

nition at the species level were evident: *T. variegatum*, *T. appendiculatum*, and *T. polyodon*. In addition to traits described above, we identify one previously known and one novel character to aid in distinguishing these species.

METHODS

Specimens representing the entire range of this group (Fig. 1) were utilized for this study from the following herbaria: ASU, BM, BRY, CAS, ISC, MU, NY, MO, PH, RENO, SD, SRP, and WIS (for herbarium acronyms, see Thiers 2015). Dried specimens were examined under a dissecting microscope. Macroscopic linear measurements and angle measurements were made using a metric ruler and protractor, respectively. Microscopic characters were measured using a Wild MC3 dissecting microscope (Wild Heerbrugg, Heerbrugg, Switzerland) and a calibrated ocular micrometer. Flowers were rehydrated using 1% Aerosol OT (Fisher Scientific, Pittsburgh, PA; Ayensu 1967). They were then dissected in order to yield measurements for corolla, calyx, stamen, and pistil characters. A total of 275 specimens yielded measurements for all characters to be used in statistical analyses (see below). The following 27 characters were used in the analyses: stem diameter, stipule lobe length, petiole length, petiole diameter, leaflet base angle, terminal leaflet length, terminal leaflet width, petiolule length, petiolule diameter, head length, flower number, peduncle length, peduncle diameter, involucre lobe length, calyx circumference, calyx lobe length, calyx lobe width, calyx tube length, calyx lobe tooth number, banner petal length, banner petal width, wing petal lamina length, wing petal lamina width, keel petal lamina length, keel petal lamina width, connate filaments length, and style length.

Principal component analysis (PCA, Pearson 1901, Hotelling 1933) was carried out using R (R Core Team, R Foundation for Statistical Computing, Vienna, Austria). PCA was performed using the *princomp* function, and projected in R. Characters selected for use in this analysis (all those listed above) represent a subset of all characters measured. They were selected for their consistency (it was easy to measure the same object at the same developmental stage for all specimens), variability (for some characters measurements were more or less invariant; these were excluded from such analyses [Sneath and Sokal 1973]), and their assumed developmental independence (logically correlated characters were left out, those highly correlated but of different organs remained in the analysis [Sneath and Sokal 1973]). Maps were produced in R (R Core Team 2013) using the packages *maps* and *mapdata*.

RESULTS AND DISCUSSION

The multivariate analysis (Fig. 2) indicates the presence of two groups. In the projection of principle

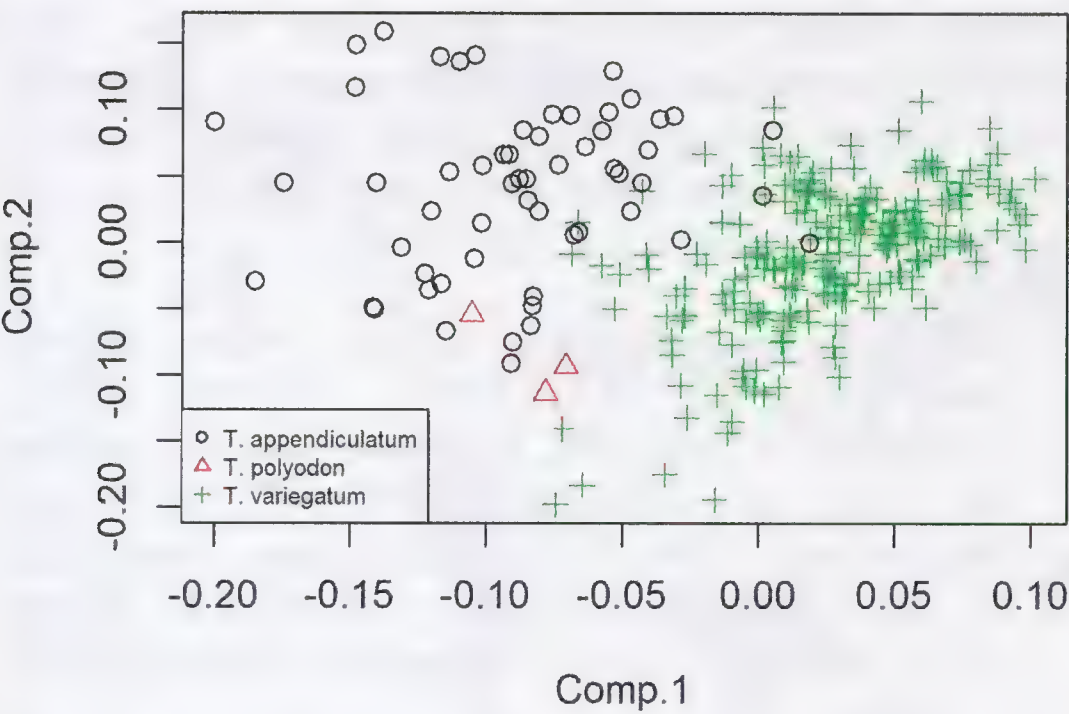


FIG. 2. Principal components analysis projection of all *T. variegatum* complex. Operational taxonomic units (OTU's) are labeled according to recognized species. Principle Component 1 (Comp. 1) explains 50.9% of variation, Principle Component 2 (Comp. 2) 11.2%.

component (PC) 1 and PC 2, the operational taxonomic units (OTU's) of *T. polyodon* are intermixed with those of *T. appendiculatum*. The characteristic morphological feature of *T. polyodon*, calyx lobe teeth, is included in the PCA, but has its highest loading on PC 7. The majority of OTU's have a character state of "0" for the number of calyx lobe teeth, so this character is ranked by the analysis as unimportant when considering morphological variation as a whole. A closer look at this character reveals that *T. polyodon* exceeds its relatives in terms of the number of calyx lobe teeth (*T. polyodon*: 8-14 teeth/calyx; *T. variegatum*: 1-8 teeth/calyx; *T. appendiculatum*: no teeth) and the average length of these teeth (*T. polyodon*: 0.1-3.1mm; *T. variegatum*: 0.1-1.4 mm; *T. appendiculatum*: no teeth). Thus, *T. polyodon* remains a separate species.

Disregarding *T. polyodon*, the two groups indicated in the multivariate analysis remain. The character loadings indicate a difference in general plant size. In PC 1, along which the groups are divided, every character has a loading of at least 0.1, and all loadings are oriented in the same direction. Thus, plants on the larger end of the spectrum are grouped into *T. appendiculatum*, while those smaller are called *T. variegatum*. The floral characters considered on their own depict a clearer separation, with *T. appendiculatum* having larger floral features, especially those of the corolla, than *T. variegatum*. In comparison, the banner petals of *T. appendiculatum* range from 6.3 to 15.8 mm in length, while those of *T. variegatum* range from 3.2 to 9.7 mm.

There are several other characters, not used in the multivariate analysis, that segregate *T. appendiculatum* from *T. variegatum*. A number (approximately 30%) of the specimens of *T. appendiculatum* presented a unique floral trait. The keel petals of these specimens have an appendage (hereafter referred to as a "beak") at their apices extending a few tenths of a millimeter. This beak, in fact, is the reason Lojacono gave *T. appendiculatum* its name (1883), and has been noticed by many botanists (e.g., Greene 1895; Heller 1905).

Trifolium appendiculatum, as circumscribed by this paper, sometimes but not always bears this morphological feature. The beak, however, never occurs in *T. variegatum*. The full merits of this trait are not yet well understood. There is no intra-individual variation of the beak. There is no as-of-yet discernable distributional pattern, and there can be both beaked and not-beaked specimens in the same county. Unfortunately, this study has not been able to investigate its heritability nor its function.

Seed size provides compelling support for recognizing these species as distinct, as has been seen in other species complexes in the genus (see, for example, seed size comparisons of *T. barbigerum* Torrey and A. Gray, *T. grayi* Lojacono, and *T. jokerstii* Vincent & R. Morgan [Vincent and Morgan 1998]). Since seeds are not present on all specimens, it was not possible to use this trait in the morphometric analyses. During an attempted common garden experiment utilizing seed from the USDA seed bank, several seeds were found to be noticeably larger than others. After propagation, plants grown from these larger seeds also bore the floral characters of *T. appendiculatum*, including a beaked keel petal. The smaller seeds gave rise to plants with the morphology of *T. variegatum*. The putative *T. appendiculatum* germplasm (USDA PI 593317) has been used in genetic analyses and appears to be genetically differentiated from *T. variegatum* (Ellison, personal communication). A sampling of seed sizes yielded a distinct separation between species ($P < 0.001$, $n=16$, Welch Two Sample t-test, $t=9.9278$), without any overlap of range.

KEY TO *TRIFOLIUM* SPECIES

- 1. Calyx lobes many-toothed, 0-4 (2.6 average) teeth per lobe, teeth 0.8 mm long on average; distal stipules 8-10 mm long; Monterey Co., CA 1. *T. polyodon*
- 1'. Calyx lobes entire, if toothed, 0-3 (0.1 average) teeth per lobe, teeth 0.3 mm long on average; distal stipules 2-10 mm long; western United States, southwestern British Columbia, Canada, and northern Baja California, Mexico 2
- 2. Banner petals 6.3-15.8 mm long, exerted 1.5-8.4 mm beyond tips of calyx lobes; mature seeds 1.9-2.8 mm long; keel petals beaked or not; California and southern Oregon..... 2. *T. appendiculatum*
- 2'. Banner petals 3.2-9.7 mm long, included in or exerted 0.1-5.7 mm beyond tips of calyx lobes; mature seeds 1.1-1.6 mm long; keel petals never beaked; British Columbia, Canada, south through Washington, Oregon, Idaho, Montana, California, Nevada, Utah, and Arizona, USA, to Baja California, Mexico 3. *T. variegatum*

TAXONOMIC TREATMENT

- 1. **Trifolium polyodon** Greene, Pittonia 3(17): 215. 1897. *Trifolium tridentatum* var. *polyodon* (Greene) Jeps., Fl. Calif. [Jepson] 2: 292. 1936. — TYPE: USA, CA: Monterey Co., Pacific

Grove, 27 May 1895, *E.L. Greene s.n.* (LECTOTYPE (here designated): NDG 67254!; isoelectotypes: NDG 67253!, NDG 67260!) (Fig. 3).

Plants annual, entirely glabrous; roots a taproot, nodulated; stems ascending to decumbent, 10–52 cm in length, 1–2.3 mm in diameter; leaves trifoliolate, dimorphic with gradual transition between proximal and distal leaves; stipules adnate to petiole, free portion lacinate, usually with one tooth longer than the rest; proximal stipules browning with age, each lobe rectangular proximally and triangular distally, 6–14×2–3 mm, margins +/- entire proximally, lacinate distally, apices acute with 1–2 lobes, the longest lobe 2–3.1 mm; distal stipules quarter-circled to ovate, recurved, 4–7×5–6 mm, margins lacinate, with 0–3 apical lobes, the longest lobe 2.5–3.2 mm; petioles 9–55×0.2–0.9 mm; petiolules 0.4–0.7× ≤0.4 mm; proximal leaflets obovate-obcordate, bases cuneate, margins serrate, apices emarginate-mucronate, terminal leaflets 4–10×4–6 mm; distal leaflets elliptic-obovate, bases cuneate, margins serrate, apices rounded, terminal leaflets 13–18.7×6.4–9.2 mm; peduncles 13–54×0.3–0.8 mm; inflorescences axillary, racemose-umbellate, involucrate, 8.2–16×10–17 mm; involucre flat, lacinate, 4.3–6.7 mm radius, lobes indistinguishable from teeth, longest tooth 1.7–2.7 mm; flowers 12–22, in 14 whorls; pedicels 0.5–1× <0.5 mm; calyces 5–7 mm long, tubes 2.3–2.9 mm in circumference, 1.9–2.4 mm long, lobes awl-like to shouldered, tips purple, 3.1–4.6 mm long, apices acute, each bearing 0–4 teeth 0.1–3.1 mm long; petals pink-purple with paler to white tips; banner petals obovate, apices emarginate, 8.3–8.8×2.1–3.2 mm, exerted beyond the tips of calyces 1.5–3.6 mm; wing petals 7.5–8.4 mm long, asymmetrically clawed, claws 4–4.5× ≤0.1 mm, laminae elongate-elliptical, 3.2–4.4×1–1.4 mm, bases shortly auriculate, auricles rounded, petal margins entire, apices rounded; keel petals 6.1–7.1 mm long, asymmetrically clawed, claws 4.4–4.8× ≤0.1 mm; laminae ovate, 1.7–2.4×1–1.3 mm, base rounded, margins entire, apices rounded; stamens diadelphous, connate filaments 6–6.6× ≤0.5 mm; pistils sessile, ovaries 2.3–2.5×0.6–0.7 mm, styles 3.6–4.6× ≤0.1 mm, ovules 2; legumes laterally compressed, 4×2 mm; seeds 2, globular to mitten-shaped, blackish-brown, dark-speckled, 1.9×1.1 mm. 2n=?

Specimens have been collected from two localities (Fig. 1): Pacific Grove (the type locality) and Indian Village, which is approximately three miles away. It has been found in wet grassy locations. All these collections were made at about 120 m in elevation. It flowers in April, May, and June.

In his Ph.D. Thesis, Martin (1943) proposed a new combination, "*Trifolium variegatum* Nutt. var. *polyodon* (Greene) Martin", but this combination was apparently never published.

Representative Specimens. USA. CALIFORNIA: Monterey Co. Pacific Grove, 21 May 1903, *Heller 6759* (ISC); Pacific Grove, 1 May 1909, *Heller 28022*

(WIS); Pacific Grove, 9 May 1909, *Heller 6707* (WIS); horticulturally grown specimens, originally from Indian Village, 20 June 1994, *Yadon s.n.* (MU); Indian Village near Bird Rock, off 17-Mile Drive, 9 April 1998, *Vincent et al. 8195* (MU).

2. ***Trifolium appendiculatum*** Lojac., Nuovo Giorn. Bot. Ital. 15: 181. 1883. —TYPE: USA, California: Coast field, *Lemmon s.n.* (not located at PAL or elsewhere); NEOTYPE (here designated): USA, California: Contra Costa Co., St. Mary's College, 14 May 1933, *J.T. Howell 11227* (neotype: CAS!; isoneotype: WIS!) (Fig. 4).

Trifolium variegatum var. *major* Lojac., Nuovo Giorn. Bot. Ital. 15: 183. 1883. —*Trifolium variegatum* var. *melananthum* f. *major* (Lojac.) McDermott, Ill. Key Amer. Trifolium 76. 1910. —TYPE: USA, California: *Lemmon s.n.* (not located at PAL or elsewhere); NEOTYPE (here designated): USA, California: Tulare Co., hills east of Yokohl Valley, 9 May 1969, *E.C. Twisselmann 15246* (neotype: CAS!; isoneotypes: SBB, RSA).

Trifolium trilobatum Jeps., Bull. Torrey Bot. Club 18: 322. 1891. —*Trifolium variegatum* var. *trilobatum* (Jeps.) Jeps. in McDermott, Ill. Key Amer. Trifolium 73. 1910. —TYPE: USA, California: Sutter Co., base of South Peak, 20 April 1891, *W.L. Jepson 14854* (holotype: JEPS - electronic image!)

Trifolium rostratum Greene, Proc. Acad. Nat. Sci. Philadelphia 47: 547. 1895. —*Trifolium appendiculatum* f. *rostratum* (Greene) McDermott, Ill. Key Amer. Trifolium 92. 1910. —*Trifolium appendiculatum* var. *rostratum* (Greene) Jeps., Man. Fl. Pl. Calif. 539. 1925. —*Trifolium variegatum* var. *rostratum* (Greene) C.L.Hitch. Vasc. Pl. Pacif. N. W. 3: 370. 1961. —TYPE: USA, California: Alameda Co., Oakland, Lake Merritt, 1889, *V.K. Chestnut s.n.* (LECTOTYPE (here designated): NDG!).

Trifolium morleyanum Greene, Erythea 3: 47. 1895. —*Trifolium variegatum* var. *melananthum* f. *morleyanum* (Greene) McDermott, Ill. Key Amer. Trifolium 76. 1910. —TYPE: USA, California: Modoc Co., Morley's Station, 1894, *Baker & Nutting s.n.* (LECTOTYPE (here designated): NDG!).

Trifolium calophyllum Greene, Pittonia 3: 213. 1897. —TYPE: USA, California: Alameda Co., Berkeley, Botanic Garden, 1895, *E.L. Greene s.n.* (lectotype (designated by Gillett 1966): NDG 67170!; isoelectotypes NDG 67171!, NDG 67172!, NDG 67214!, NDG 67176!).

Trifolium phaeocephalum Greene, Pittonia 3: 216. 1897. —*Trifolium variegatum* var. *pauciflorum* f. *phaeocephalum* (Greene) McDermott, Ill. Key Amer. Trifolium 78. 1910. —TYPE: USA, California: Butte Co., east of Chico, May 1883, *R.M. Austin s.n.* (lectotype (designated by Gillett 1966): NDG!).

Trifolium splendens A.Heller, Muhlenbergia 1: 115. 1905. —TYPE: USA, California: Monterey Co., Pacific Grove, 7 May 1903, *A. Heller 6691* (holotype: BKL; isotypes: AC - electronic image!,



FIG. 3. Specimen of *T. polyodon*. This horticulturally grown specimen, *Yadon s.n.* (MU), depicts the phenotypic plasticity possible within a population. The calyx lobe teeth are not visible without magnification.

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BATON ROUGE

37637



HERBARIUM MU
Trifolium appendiculatum Lojac.
Det.: B.G. Foster & M.A. Vincent 2015

HERBARIUM MU
Trifolium variegatum Nuttall *sl.*
Det.: Michael A. Vincent 1998

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HERBARIUM
CALIFORNIA STATE COLLEGE AT LOS ANGELES
Trifolium variegatum Nutt. det RMS
Petal tips light purple 7.5P 7/7, base of
flower deep purplish red 7.5RP 3/9.
CALIFORNIA: Tulare Co.: Yokohl Valley road,
17 miles east of Exeter; oak woodland, pastured
425 m elev.
R. M. Straw 2620
Date: 8 May 1965 Collected by: B. Miller, M. Kamin
(82017 67)

FIG. 4. Specimen of *T. appendiculatum*. This specimen, *R.M. Straw, B. Miller, and M. Kamin 2620* (MU), has flowers with beaked keel petals, but these are not visible without magnification. The long banner petals exceeding the calyx lobes, however, are apparent.

BM!, CAS - electronic image! (3 sheets), F - electronic image!, G - electronic image!, ISC!, K, MICH - electronic image!, MSC - electronic image!, NY - electronic image! (two sheets), P - electronic image!, PH (2 sheets)!, US - electronic image!, WIS!)

Plants annual, entirely glabrous; roots a taproot, nodulated; stems ascending, 7–82 cm in length, 0.5–3 mm in diameter; leaves trifoliate, dimorphic with gradual transition between proximal and distal leaves; stipules adnate to petiole, free portion lacinate, usually with one tooth longer than the rest; proximal stipules browning with age, each lobe rectangular proximally and triangular distally, 7–13×2–5 mm, margins +/- entire proximally, lacinate distally, apices acute with 1–2 lobes, the longest lobe 1.5–3 mm; distal stipules quarter-circled to ovate, recurved, 3.4–10.3×5–6 mm, margins lacinate, with 0–many apical lobes, the longest lobe 0.8–4 mm; petioles 2–73×0.2–1 mm; petiolules 0.3–0.9×≤0.5 mm; proximal leaflets obovate-obcordate, bases cuneate, margins serrate, apices emarginate-mucronate, terminal leaflets 2.9–7×1.5–5 mm; distal leaflets elliptic-obovate, bases cuneate, margins serrate, apices rounded and mucronate, terminal leaflets 5.8–25.9×2.8–10.9 mm; peduncles 17–73×0.3–1.3 mm; inflorescences axillary, racemose-umbellate, involucre, 7.3–21×8–31 mm; involucre flat, lacinate, 3.2–6.5 mm radius, 4–7 lobes, lobes at time indistinguishable from teeth, longest tooth 1.1–3 mm; flowers 4–25, in 1–4 whorls; pedicels 0.3–0.5×<0.5 mm; calyces 3.5–7.4 mm long, tubes 2.2–4.6 mm in circumference, 1.5–3.3 mm long, lobes awl-like to shouldered, tips and shoulders purple, 2.2–5.2 mm long, apices acute, usually toothless, teeth < 1 mm; petals deep purple, white-tipped; banner petals obovate, apices emarginate, 6.3–15.8×1.5–4 mm, exerted beyond the tips of calyces 1.5–8.4 mm; wing petals 5.3–14.2 mm long, asymmetrically clawed, claws 2.9–7×≤0.1 mm, laminae elongate-elliptical, 3–7.3×0.8–1.9 mm, bases shortly auriculate, auricles rounded, petal margins entire, apices rounded; keel petals 4.6–10.6 mm long, asymmetrically clawed, claws 2.8–6.7×≤0.1 mm, laminae ovate, 1.8–3.9×0.8–1.8 mm, base rounded, margins entire, apices rounded to beaked, beaks ≤ 1 mm; stamens diadelphous, connate filaments 3.9–9.8×≤0.5 mm; pistils sessile, ovaries 1.4–4.1×0.3–1.1 mm, styles 2.4–6.2×≤0.3 mm, ovules 2; legumes laterally compressed, 3.6–3.8×2–2.4 mm; seeds 2, globular to mitten-shaped, blackish-brown, black-speckled, 1.9–2.8×1.2–2 mm. 2n=?

Trifolium appendiculatum is found throughout California (Fig. 1), though never east of the Sierra Nevada Mountains, and into southern Oregon (one locality; it is likely rare in Oregon, but our study had poor coverage for this state). It is often collected in open fields, meadows, ditches, marshes, swamps, oak woodlands, and digger pine woodlands, and in rocky, sandy, or loamy soil. Its elevation ranges from 0 to 1400 m. It flowers in March, April, May, and June.

Representative Specimens. **USA. CALIFORNIA:** Butte Co., hills near Big Chico Creek east of Chico, 6 April 1913, *Heller 10710* (CAS); Kern Co., high slope 1 mile east of Poso Creek Narrows, 17 May 1963, *Twisselmann 8251* (CAS); Monterey Co., west of S. Fran., June 1876, *Palmer 71* (GH); San Diego Co., Cuyamaca Rancho State Park. Approx. 0.4 mile NE of intersection of State hwy., 26 June 2005, *Hendrickson 1084* (SD); San Joaquin Co., eastern edge of Wallace, 2 May 1959, *Smith 1063* (CAS); Sutter Co., found growing on damp soil along the edge of West Butte Road, about 1/8 mile north of the Sacramento Outing Club, Sutter Buttes, 19 April 1984, *Ahart 4566* (CAS). **OREGON:** Josephine Co., Grant's Pass, 16 May 1910, *Heller 10030* (CAS), *Heller 10031* (ISC, CAS [2 sheets]).

3. *Trifolium variegatum* Nutt. in Torr. & A. Gray, *Flora of North America* 1: 317. 1838. —TYPE: USA, Oregon: springy places near the mouth of the Wahlamet, *T. Nuttall s.n.* (LECTOTYPE (here designated): NY - electronic image!; isolectotypes: BM!, GH, K) (Fig. 5).

Trifolium spinulosum var. *triste* Torr. & A. Gray, *Fl. N. Amer.* 1: 318. 1838. TYPE: USA, California: Santa Barbara Co., Santa Barbara, *T. Nuttall s.n.* (LECTOTYPE (here designated): BM!).

Trifolium melananthum Hook. & Arn., *Botany of Captain Beechey's Voyage* 331. 1838. —*Trifolium variegatum* var. *melananthum* (Hook. & Arn.) Greene, *Fl. Francisc.* 1: 29. 1891. —*Trifolium tridentatum* var. *melananthum* (Hook. & Arn.) S. Watson, *Proc. Amer. Acad. Arts* 11: 130. 1876. —TYPE: USA, California: *D. Douglas s.n.* (LECTOTYPE (here designated): K 001051289 - electronic image!; isolectotype: BM 010892 - photocopy!).

Trifolium dianthum Greene, *Pittonia* 3: 217. 1897. —TYPE: Canada, British Columbia: Vancouver Island, vicinity of Victoria, 13 May 1893, *J. Macoun 94* (LECTOTYPE (here designated): NDG 67320!; isolectotype: MO!).

Trifolium geminiflorum Greene, *Pittonia* 3: 216. 1897. —*Trifolium variegatum* var. *geminiflorum* (Greene) Vincent, *Madroño* 56: 208. 2009. —TYPE: USA, California: Shasta Co., Lassen's Peak, August 1879, *R. M. Austin s.n.* (LECTOTYPE (here designated): NDG 67301A!; syntype: USA, California: Nevada Co., Donner Lake, July 1890, *C.F. Sonne 3* (NDG 67301B!); syntype: USA, California: Nevada Co., Donner Lake, August 1890, *C.F. Sonne 5* (NDG 67301C!); syntype: USA, California, Amador Co., 1891, *Hansen s.n.* (NDG 67208!).

Trifolium pusillum Greene, *Pittonia* 3: 217. 1897. —TYPE: USA, California: Yosemite Valley, 1881, *C.C. Parry 45* (LECTOTYPE (here designated): NDG!; isolectotype: MO!).

Trifolium subsalinum Greene, *Pittonia* 3: 219. 1897. —TYPE: USA, Nevada: Eureka Co., Palisade, 24 July 1893, *E.L. Greene s.n.* (lectotype (designated by Gillett 1966): NDG!; syntype: USA, Nevada:

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HERBARIUM MU
Trifolium variegatum Nutt.

Det.: B.G. Foster & M.A. Vincent 2015

CALIFORNIA

Trifolium variegatum Nutt.

Mendocino Co., road to Dos Rios from Laytonville.

Stream bed, common but not in dry areas.

J.M. Gillett 12951 June 8, 1966
C.W. Crompton

Chromosome number from
~~this plant~~
this collection $n=8x$

Determined by D. E. Hayley 1966
Annotated by
Department of Agriculture, Ottawa.

DEPARTMENT OF AGRICULTURE, OTTAWA, CANADA

RB 100

FIG. 5. Specimen of *T. variegatum*. This specimen, J.M. Gillett and C.W. Crompton 12951 (MU), depicts a typical example of the species.

Eureka Co., Palisade, 24 August 1896, *E.L. Greene s.n.* (NDG!).

Trifolium ultramontanum Greene, Pittonia 3: 218. 1897. —TYPE: USA, Nevada: Elko Co., Holborn, 16 July 1896, *E.L. Greene s.n.* (lectotype (designated by Gillett 1966): NDG 67201!; isolectotype: NDG 67202!, NDG 67204!); syntype: USA, Nevada: Star Valley, 20 July 1896, *E.L. Greene s.n.* (NDG!).

Plants annual, entirely glabrous; roots a taproot, nodulated; stems prostrate to erect, 2.5–48+ cm in length, 0.1–2.3 mm in diameter; leaves trifoliate, dimorphic, with gradual or abrupt transition between proximal and distal leaves; stipules adnate to petiole, free portion laciniate, usually with one tooth longer than the rest; proximal stipules browning with age, each lobe rectangular proximally and triangular distally, 2.4–10×0.5–2.1 mm, margins +/- entire proximally, laciniate distally, apices acute with 1–2 lobes, the longest lobe 1–2.5 mm; distal stipules quarter-circled to ovate, recurved or not, 1.7–9.3×0.5–2.5 mm, margins laciniate, with 0–3 apical lobes, the longest lobe 0.6–4.5 mm; petioles 1–95×0.1–1.1 mm; petiolules 0.1–0.8×≤0.5 mm; proximal leaflets obovate-obcordate-cuneate, bases cuneate, margins serrate, apices emarginate-mucronate, terminal leaflets 1.9–5×0.9–2.1 mm; distal leaflets elliptic-obovate, bases cuneate, margins serrate, apices rounded and mucronate, terminal leaflets 2–29×1–11.4 mm; peduncles 4–53×0.1–0.7 mm; inflorescences axillary, racemose-umbellate, involucre, 4.6–12.6×0.8–10 mm; involucre flat, laciniate, 1.5–6 mm radius, 2–7 lobes, at times indistinguishable from teeth, longest tooth 0.6–3 mm; flowers 1–25, in 1–4 whorls; pedicels 0.1–0.5×<0.1 mm; calyces 2.1–6.8 mm long, tubes 1.4–4.1 mm in circumference, 1.9–2.4 mm long, lobes awl-like to shouldered, tips purple or stramineous, 1.5–4.8 mm long, apices acute, occasionally bearing up to 3 teeth per lobe, teeth < 0.6 mm long; petals purple, usually white-tipped; banner petals obovate, apices emarginate, 3.2–9.7×0.6–2.6 mm, included in or exerted beyond the tips of calyces 0.1–5.7 mm; wing petals 3.1–9 mm long, asymmetrically clawed, claws 1.6–5.1×≤0.1 mm, laminae elongate-elliptical, 1.3–4×0.2–1.4 mm, bases shortly auriculate, auricles rounded, petal margins entire, apices rounded; keel petals 3–7.3 mm long, asymmetrically clawed, claws 1.4–4.8×≤0.1 mm, laminae ovate, 12.5×0.4–1.8 mm, base rounded, margins entire, apices rounded-short tipped; stamens diadelphous, connate filaments 2.2–6.6×≤0.5 mm; pistils sessile, ovaries 2–2.9×0.2–1.4 mm, styles 1.1–4.2×≤0.1 mm, ovules 2; legumes laterally compressed, 2.2–4×1.3–2 mm; seeds 2, globular to mitten-shaped, blackish-brown, dark-speckled or not, 1.1–1.6×1–1.2 mm. 2n=16.

Trifolium variegatum is the most widespread of the three species studied, encompassing the ranges of the other two (Fig. 1). It does not appear to have any unique habitat requirements other than some moisture. It is found in grasslands, sandy stream beds, and other open areas, but can also be found in

forests. It occurs in elevations ranging from sea level to 3,000 m. It has been collected in every month from March to October, although this varies depending on the geographic area.

While Gillett (1966) lectotypified a number of Greene's *Trifolium* spp., he did not do so for *T. geminiflorum*. Gillett writes that *T. geminiflorum* Greene was a new name for *T. pauciflorum sensu* Lojac. and then goes on to cite a Lemmon specimen referred to by Lojacono. However, Lojacono (1883) was merely commenting on what he thought was a strange specimen (collected by Lemmon) of Nuttall's *T. pauciflorum* and neither intended nor effected a newly published name. Thus, *T. geminiflorum* remained to be lectotypified. It was incorrectly lectotypified by Vincent (2009) because the specimen selected as lectotype (*G. Hansen 1*, Amador Co., April 1892, cited as at NDG but not indicated as having been seen) does not exist at NDG. There is a Hansen specimen (without a number) from 1891 (NDG67208) at NDG, and there are specimens of *Hansen 1* at BM and K (the one at Kew was selected by Vincent as isolectotype). Since, however, *Hansen 1* does not exist at NDG, it is unlikely that Greene saw it, and instead was referring to *Hansen s.n.* (1891, Amador Co.; listed as a syntype above). Thus, Vincent's (2009) incorrect lectotypification is set aside and replaced by the one presented here.

Representative Specimens. **CANADA. BRITISH COLUMBIA:** Vancouver Island, very common especially in wet areas on rocky-grassy bluffs above river, Sproat River Falls north of Alberni, 7 June 1961, *Calder and MacKay 30139* (CAS); Victoria Three miles northeast of Duncan on road to Maple Bay, 19 June 1961, *Calder and MacKay 30732* (CAS). **MEXICO. BAJA CALIFORNIA:** Ensenada: Sierra San Pedro Martir, meadows along road heading south of Vallecitos towards La Encantada, base of Cerro Botella Azul, 19 July 1988, *Boyd et al. 2683* (MU); Laguna Hanson, Constitucion National park, Sierra de Juarez; north end of lake on sandy marsh delta and wet margins of lake, 28 May 1983, *Thorne et al. 55739* (WIS). **USA. ARIZONA:** Graham Co.: Coronado National Forest, Holdout Creek, just above jcn. with Black Rock wash along trail splitting off by corrals, 10 June 1998, *Buegge et al. 338* (ASU). **CALIFORNIA:** San Bernardino Co., Vicinity of Bonanza King Mine, East Slope of Providence Mountains, Mojave Desert, 21 May 1920, *Munz et al. 4020* (MU, CAS); Santa Barbara Co., Santa Cruz Island, Creek below Main Ranch (toward Prisoner's Harbor), 12 October 1958, *Balls 11820* (BM); Inyo Co., Wild Rose Canyon, Panamint Mts., 30 June 1931, *Hoffmann s.n.* (CAS); Tuolumne Co., Tioga Road from Harden Lake to Pareuphene Flat, Yosemite National Park, 13 August 1907, *Eastwood 317* (CAS); San Diego Co., Cleveland National Forest: Northeast of El Cajon Mountain, west of the San Diego River, and south of Barona Mesa, just off of El Cajon Mountain Truck Trail, 7 April 2010, *Rebman et al. 18976* (SD); Kern Co.,

Saddle Springs, north end of the crest of Piute Mountain, 22 June 1962, *Twisselmann 7349* (CAS); Monterey Co., “The Indians” near Jolon, 6 April 1988, *Morgan 1065* (MU). **IDAHO:** Blain Co., Tikura, 22 July 1911, *Nelson and Macbride 1303* (CAS); Kootenai Co., June 1889, *Sandberg s.n.* (MU); Owyhee Co.: S Quicksilver Mountain above Boone Peak, 19 July 1996, *Atwood 21000* (MU, BRY). **MONTANA:** Missoula Co., Missoula, Hay Spur, Geo. F. Fox Ranch, 19 June 1924, *Kirkwood 1756* (MU, ISC, CAS [2 sheets]). **NEVADA:** Nye Co., Along Eden Creek, 2 mi. east on road past Eden Ranch, about 15 mi. due south of Warm Springs, 28 August 1980, *Neese and White 9744* (BRY); Elko Co., Deeth, 23 July 1908, *Heller 9129* (ISC); Washoe Co.: Above the reservoir on Heinz Ranch, northwest slope of Peavine Mountain about 10 miles north of Reno, 21 September 1974, *Howell et al. 50973* (CAS). **OREGON:** Wasco Co., Dalles City, June 8 1897, *Suksdorf 2583* (GH); Lane Co., West of Science building Lane Community College, 9 May 1992, *Love 930* (MU); Clackamas Co., Gladstone, May 1894, *Howell 135* (WIS); Coos Co., 2 mi. west of Charleston opposite Rhodo-Azalea Gds. Nursery, 29 June 1961, *Kimber 122* (WIS). **UTAH:** Box Elder Co., southwest corner of the county, 16 mi. south of Lucine, east of the Pilot Range, Patterson Spring at edge of Great Salt Lake Desert in greasewood belt, 5 July 1976, *Arnow 5013* (BRY, MU); Salt Lake Co., Salt Lake City, July 1880, *Howard s.n.* (BRY). **WASHINGTON:** Ferry Co., Open, springy, gravelly spot 1/4 mi. west of Columbia River, Kettle Falls, 17 June 1939, *Weldert and Boner 151* (CAS [2 sheets]); San Juan Co., Lopez Island, 17 July 1937, *Evans 11524* (MU); Cowlitz Co., Moist bluffs along the Columbia River near Kalama, 7 April 1934, *Thompson 10129* (CAS).

Excluded Taxon

Trifolium variegatum var. *parunuweapensis* S.L. Welsh, Utah Fl., ed. 3. 425. 2003. —TYPE: USA, Utah: Kane Co., ca. 1.5 mi S of The Barracks, subsequent drainage of Parunuweap Canyon, 1450m, 16 July 1992, *S.L. Welsh & K.H. Thorne 25120* (holotype: BRY!).

After comparing the type specimen of *T. variegatum* var. *parunuweapensis* to the type specimen of *T. mucronatum* subsp. *lacerum* (Greene) Gillett (holotype: US - electronic image!, isotype: NY - electronic image!; sometimes synonymized with *T. wormskioldii*), it is evident that this variety is a synonym of *T. mucronatum* subsp. *lacerum*. *Trifolium variegatum* var. *parunuweapensis*, like *T. mucronatum* subsp. *lacerum*, produces rhizomes (visible on paratypes, difficult to distinguish on holotype), has stipules up to 30 mm long, and has three or four ovules. None of these features are ever found in *T. variegatum*, *T. polyodon*, or *T. appendiculatum*.

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MADROÑO

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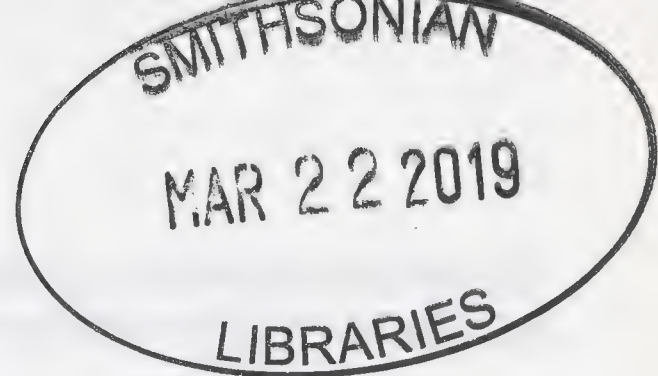
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NOTEWORTHY COLLECTION

BAJA CALIFORNIA

The following 11 range extensions and new records are from collections made during an expedition in April 2017 to an area with several vernal lakes in the Central Desert ecoregion in the central portion of the state of Baja California, but are mostly plants not directly associated with the vegetation of this ecoregion or the vernal lakes. Most of these records are significant southward range extensions of plants from the California Floristic Province and extend their range into the Baja California Central Desert ecoregion. The collection of *Eragrostis barrelieri* Daveau is a new record for the state of Baja California, the collection of *Malacothrix coulteri* Harv. & A. Gray is the second record from the state with the previous one collected in 1882, and the collection of *Euphorbia petrina* S. Watson extends its range from the Lower Colorado Desert ecoregion to the Pacific drainage and the Central Desert. The previous distributional knowledge of species listed in these accounts is based on georeferenced specimens from various herbaria that are in the combined database of the Baja California Botanical Consortium, available on the website bajaflora.org (Baja-Flora 2018).

ARTEMISIA CALIFORNICA Less. (ASTERACEAE). Ensenada Mpio. (Municipio). Canyon draining into Laguna El Caporal from the northwest, west of Laguna Chapala and Laguna El Islote. 29.26960° N, 114.59020° W, elevation 365 m, 23 April 2017, *Rebman 33109* (SD). Growing in lower part of the canyon, primarily in alluvium from the canyon sides, which were composed of limestone on the south side and basalt on the north. Central Desert/Sage Scrub vegetation with *Aldama purisimae* (Brandege) E.E.Schill. & Panero, *Ambrosia chenopodiifolia* (Benth.) Payne, *Bahiopsis laciniata* (A. Gray) E.E.Schill. & Panero, *Encelia asperifolia* (S.F.Blake) C.Clark & Kyhos, *Fouquieria columnaris* Kellogg, *Lycium* sp., and *Pachycereus pringlei* Britton & Rose.

Previous knowledge. A well known native of the California Floristic Province and an indicator species of Coastal Sage Scrub. This species ranges from the San Francisco Bay area of California into northwestern Baja California and offshore to Cedros and Guadalupe islands.

Significance. This collection extends the known range on the Baja California peninsula 35 km south from a previous collection near Cataviña, which is also disjunct from the main populations of this species on the peninsula. It grows here with plants

typical of the Central Desert ecoregion of Baja California.

CRYPTANTHA CLEVELANDII Greene var. *CLEVELANDII* (BORAGINACEAE). Ensenada Mpio. Vicinity of a small vernal lake: east of San José de la Piedra and south of Rancho El Llano; dry wetland area 29.30434° N, 114.66959° W, elevation 420 m, 23 April 2017, *Rebman 33139* (SD). Central Desert vernal lake vegetation with *Aldama purisimae*, *Amblyopappus pusillus* Hook. & Arn., *Ambrosia chenopodiifolia*, *Bromus rubens* L., *Deinandra fasciculata*, *Lasthenia gracilis* (DC.) Greene, *Lycium californicum* Nutt. ex A.Gray, and *Marsilea vestita* Hook. & Grev.

Previous knowledge. A native coastal species of California and northwestern Baja California as far south as the San Quintín area.

Significance. This is a range extension of 180 km to the SSE and is the first record of *Cryptantha clevelandii* var. *clevelandii* in the Central Desert ecoregion.

DISTICHLIS BAJAENSIS H.L.Bell (POACEAE). Ensenada Mpio. Arroyo San José: east of San José de la Piedra and southwest of Rancho El Llano; saline flood plain area in arroyo. 29.32242° N, 114.77581° W, elevation 40 m, 24 April 2017, *Rebman 33180* (SD). Riparian vegetation with *Atriplex julacea* S. Watson, *Distichlis bajaensis*, *Distichlis spicata* (L.) Greene, *Frankenia palmeri* S.Watson, *Isocoma acradenia* (Greene) Greene, *Iva hayesiana* A.Gray, *Juncus acutus* L., *Prosopis glandulosa* var. *torreyana* (L.D.Benson) M.C.Johnst., *Suaeda nigra* (Raf.) J.F.Macbr., and *Tamarix ramosissima* Ledeb.

Previous knowledge. This is thought to be a very rare grass originally described from a single population (Bell 2010), now known from only four populations in the state of Baja California, ranging along the west coast from near Colonet to near Santa Rosalillita.

Significance. This adds a fifth population to the known occurrences of this species. This population is quite large, extending along the bottom of the arroyo for more than a kilometer. It seems likely that more populations of this species may exist in other arroyos in west-central Baja California.

ERAGROSTIS BARRELIERI Daveau (POACEAE). Ensenada Mpio. South of Miller's Landing and Rosarito and north of Villa Jesús María; along Mex. Hwy. 1; sandy, saline shoulder of the highway. 28.40666° N, 113.98971° W, elevation 37 m, 21 April 2017, *Rebman 33076* (SD). Ruderal vegetation with

Astragalus fastidius, *Chenopodium murale*, *Malva parviflora*, and *Mesembryanthemum crystallinum*.

Previous knowledge. This non-native, southern European, annual grass was introduced to Sonora and is now scattered in Mexico, the southern U.S., and southwestern California. It is known rarely from the state of Baja California Sur.

Significance. This is the first collection of *Eragrostis barrelieri* from the state of Baja California and the first record of it in the Central Desert ecoregion.

ERIASTRUM FILIFOLIUM (Nutt.) Woot. & Standl. (POLEMONIACEAE). Ensenada Mpio. Northwest end of Laguna El Caporal: west of Laguna El Islote and Laguna Chapala; in a small canyon on the northwest side of the dried lake and on lower slopes of the limestone cliffs of the canyon. 29.26960° N, 114.59022° W, elevation 365 m, 23 April 2017, *Rebman 33119* (SD). Central Desert/Sage Scrub vegetation with *Aldama purisimae*, *Ambrosia chenopodiifolia*, *Artemisia californica*, *Bahiopsis laciniata*, *Encelia asperifolia*, *Fouquieria columnaris*, *Lycium* sp., and *Pachycereus pringlei*.

Previous knowledge. This species is known from northwestern Baja California extending as far south as the area of El Rosario and north into coastal Southern California (Rebman et al. 2016).

Significance. This collection extends the range 115 km to the SSE, well into the Central Desert ecoregion of Baja California.

EUPHORBIA PETRINA S.Watson. (EUPHORBIA-CEAE). Ensenada Mpio. South of Miller's Landing and Rosarito and north of Villa Jesús María; along Mex. Hwy. 1; sandy, saline shoulder of the highway. 28.40666° N, 113.98970° W, elevation 37 m, 21 April 2017, *Rebman 33077* (SD). Ruderal vegetation with *Astragalus fastidius* M.E.Jones, *Chenopodium murale* L., *Malva parviflora* L., and *Mesembryanthemum crystallinum* L.

Previous knowledge. An uncommon native in Baja California, found in the Lower Colorado Desert ecoregion of northeastern Baja California, and also found on various islands in the Gulf of California as far south as Isla Partida off Baja California Sur. It is also found in Sonora and Sinaloa on the Mexican mainland.

Significance. This is a range extension of 212 km. south on the mainland of the peninsula from a specimen collection near San Felipe. It is the first collection from the Central Desert ecoregion and the first from the Pacific drainage in Baja California.

FRAXINUS PARRYI Moran. (OLEACEAE). Ensenada Mpio. Northwest end of Laguna El Caporal: west of Laguna El Islote and Laguna Chapala; in a small canyon on the northwest side of the dried lake and on lower slopes of the limestone cliffs of the

canyon. 29.26960° N, 114.59022° W, elevation 365 m, 23 April 2017, *Rebman 33116* (SD). Central Desert/Sage Scrub vegetation with *Aldama purisimae*, *Ambrosia chenopodiifolia*, *Artemisia californica*, *Bahiopsis laciniata*, *Encelia asperifolia*, *Fouquieria columnaris*, *Lycium* sp., and *Pachycereus pringlei*.

Previous knowledge. This species is a near-endemic to northern Baja California, with a small population in southern San Diego County. In northwestern Baja California it is often a dominant species in Chaparral and Coastal Sage Scrub plant communities.

Significance. This is a range extension of 116 km to the SSE from a previous collection north of El Rosario. This extends the range well into the Central Desert ecoregion. This population consists of many relatively large and healthy-looking individuals.

GILIA ANGELENSIS V.E.Grant (POLEMONIACEAE). Ensenada Mpio. Vicinity of a small vernal lake east of San José de la Piedra and south of Rancho El Llano; dry wetland area. 29.30434° N, 114.66959° W, elevation 420 m, 23 April 2017, *Rebman 33142* (SD). Central Desert vernal lake vegetation with *Aldama purisimae*, *Amblyopappus pusillus*, *Ambrosia chenopodiifolia*, *Bromus rubens*, *Deinandra fasciculata*, *Lasthenia gracilis*, *Lycium californicum*, and *Marsilea vestita*.

Previous knowledge. This native is found uncommonly in northwestern Baja California as far south as the vicinity of El Rosario (Rebman et al. 2016). It is also found as far north as the San Francisco Bay region in California.

Significance. This is a range extension of 130 km to the SSE, well into the Central Desert ecoregion of Baja California.

MALACOTHRIX COULTERI Harv. & A.Gray (ASTERACEAE). Ensenada Mpio. Vicinity of large vernal lake (Laguna Grande): east of San José de la Piedra and south of Rancho El Llano; northern side of dry lake bed. 29.33676° N, 114.64923° W, elevation 428 m, 21 April 2017, *Rebman 33066* (SD). Central desert vernal lake margin vegetation with *Ambrosia pumila* A.Gray, *Deinandra fasciculata* Greene, *Euphorbia albomarginata* Torr. & A.Gray, *Hoffmannseggia glauca* (Ortega) Eifert, and *Schismus barbatus* (L.) Juel.

Previous knowledge. A widespread native in California, found in coastal, mountain, and desert areas. Also found in other states of the southwestern USA and reported from South America. There is a single historic collection (*M.E. Jones 3708* from 1882) in the state of Baja California, from the area of Tijuana.

Significance. This is a range extension of 250 km to the SSE on the Baja California peninsula, and confirms this species' presence in the Central Desert ecoregion, where it had previously been reported

(Rebman et al. 2016), but never collected. It was seen in more than one location on this trip, and in considerable numbers.

POA SECUNDA J. Presl subsp. *SECUNDA* (POACEAE). Ensenada Mpio. Northwest end of Laguna El Caporal: west of Laguna El Islote and Laguna Chapala; in a small canyon on the northwest side of the dried lake and on lower slopes of the limestone cliffs of the canyon. 29.26960° N, 114.59022° W, elevation 365 m, 23 April 2017, *Rebman 33115* (SD). Central Desert/Sage Scrub vegetation with *Aldama purissima*, *Ambrosia chenopodiifolia*, *Artemisia californica*, *Bahiopsis laciniata*, *Encelia asperifolia*, *Fouquieria columnaris*, *Lycium* sp., and *Pachycereus pringlei*.

Previous knowledge. This widespread native perennial grass occurs in the western U.S., Canada, and South America. In Baja California it occurs on Guadalupe Island and on the peninsula in the northwestern portion as far south as Mesa San Carlos.

Significance. This is a range extension of 103 km to the southeast.

STIPA CERNUA Stebb. & Löve (POACEAE). Ensenada Mpio. Vicinity of Laguna La Guija: south of Cataviña and northwest of Laguna Chapala; west of Mex. Hwy. 1; sandy, saline flats on the eastern side of Laguna La Guija. 29.41455° N, 114.48302° W, elevation 690 m, 21 April 2017, *Rebman 33073* (SD). Central Desert vernal lake margin vegetation with *Ambrosia pumila*, *Atriplex canescens* (Pursh) Nutt., *Caulanthus lasiophyllus* (Hook. & Arn.) Payson, *Erodium cicutarium* (L.) L'Hér. ex Aiton, *Eulobus californicus* Torr. and A.Gray, and *Lycium californicum*.

Previous knowledge. This native grass is widespread in California. The known range in northwest-

ern Baja California extends south to near the Meling Ranch in the western foothills of the Sierra San Pedro Mártir.

Significance. This is a range extension of 210 km to the SSE and is the first record of *Stipa cernua* in the Central Desert ecoregion of Baja California.

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NOTEWORTHY COLLECTION

CALIFORNIA

AGARDHIELLA SUBULATA (C. Agardh) Kraft & M.J.Wynne (SOLIERIACEAE).—Alameda Co., attached to rocks, Lake Merritt, Oakland, 37.80332, –122.26088, sterile, 10 Aug 2012, *R. L. Moe s.n.* (UC 2050478, UC 2050479); Marina Village Yacht Harbor, Alameda, 37.78483, –122.269120, sterile, 17 Sep 2010, *K. Lesyna s.n.* (UC 1983203).

Previous knowledge. *Agardhiella subulata* is a marine red alga with subdichotomous branching and slender axes that bear irregular numbers of awl-shaped laterals (Kraft and Wynne 1979). It is native to the Atlantic coast of North America (type locality “*In mari Canadensi*”) (Agardh 1822). It has been reported worldwide (Guiry and Guiry 2018), but most of these reports were presented without genetic confirmation. Reports of *A. subulata* introductions include Thau Lagoon, France (Verlaque 2001), Italy (Curiel et al. 2005; Sfriso and La Rocca 2005; Manghisi et al. 2010), and the Netherlands (Stegenga 1999).

Significance. This is the first report of this species in the eastern Pacific Ocean. This species was collected from Marina Village Yacht Harbor, Alameda, CA, in 2010 and Lake Merritt, Oakland, CA, in 2012. The identification was confirmed by analyzing 1294 base pairs (bp) of *rbcL* DNA sequence data from one specimen of *A. subulata* from Alameda and two specimens from Lake Merritt (GenBank accessions MH179248, MH179249, MH179250) following the DNA extraction protocol of Lindstrom et al. (2011) and PCR methods of Hughey and Hommersand (2008). The three identical sequences differed by 3 bp from a 1404 bp sequence of *A. subulata* from North Carolina, USA (Freshwater et al. 1994; GenBank accession U04176), and by 3 bp from a 470 bp sequence of *A. subulata* isolated from introduced material that was maintained in indoor tank cultures at two fisheries institutions in Okinawa and Kagoshima Prefectures from Japan (Vo et al. 2014; GenBank accession U04176). The DNA sequences of the North Carolina and Japanese specimens were identical, and there are no other sequences in GenBank. Because the DNA sequences of the Alameda and Lake Merritt specimens do not match either of these, we cannot determine the origin of the California introduction. We know neither how long this species has been in California nor do we know its current distribution, since it strongly resembles (and can be easily confused with) the common and presumed native species, *Sarcodiotheca gaudichaudii* (Montagne) P. W. Gabrielson. A search for other specimens from

San Francisco and Tomales bays was unsuccessful; specimens that were morphologically similar to *A. subulata*, but were identified as *Sarcodiotheca gaudichaudii* (UC 1861453, UC 1965548, UC 2010034, UC 2009418), were confirmed as *S. gaudichaudii* by *rbcL* gene sequences.

COLPOMENIA CLAYTONIEAE S.M. Boo, K.M. Lee, G.Y. Cho & W. Nelson (SCYTOSIPHONACEAE).—Monterey Co., attached to sunny side of dock in Monterey Harbor, Monterey, 36.608972, –121.893528, gametophytic thalli with plurangia, 7 Feb 2018, *J. R. Hughey s.n.* (UC 2050588) and 19 Feb 2018, *J. R. Hughey s.n.* (UC 2050589).

Previous knowledge. *Colpomenia claytoniae* is a widely distributed species reported from Korea, Japan, Hong Kong, Australia, New Zealand, South Africa, and the USA (type locality: Sangjokam, Goseong, Korea) (Boo et al. 2011). On the basis of *cox3* DNA sequences, these authors suspected that some of the populations of *C. claytoniae* may be the result of recent introductions. In California, *C. claytoniae* has been confirmed from only two localities: San Pedro and Corral State Beach Park, Malibu. The thallus of *C. claytoniae* is globular and hollow, irregularly convoluted, thin, and expands up to 30 cm in diameter (Boo et al. 2011). It has been overlooked or previously, and incorrectly, identified as *C. peregrina* Sauvageau in northern California and as *C. sinuosa* (Mertens ex Roth) Derbès & Solier south of Point Conception.

Significance. This collection extends the range of *C. claytoniae* approximately 480 km north. The gametophytic thalli from Monterey are typical of this species. When fresh they are thin and grow to approximately 7 cm in height, and up to 25 cm in diameter. The Monterey specimens were attached to the dock in full sunlight, growing adjacent to the invasive brown kelp *Undaria pinnatifida* (Harvey) Suringar, which was first collected in the Monterey Marina in 2001 (Silva et al. 2002). The identification was confirmed by analyzing 548 bp of DNA sequence data of the *cox3* gene from one of the Monterey specimens of *C. claytoniae* (GenBank accession MG976804) following the DNA extraction techniques of Lindstrom et al. (2011) and PCR methods of Boo et al. (2011). The Monterey specimen was identical to four sequences of *C. claytoniae* from San Pedro, California (GenBank accessions HQ833812, HQ833813), Island Bay, Wellington, New Zealand (GenBank accession HQ833798), and Wilson Bay, Coromandel, New Zealand (GenBank accession HQ833807), but differed by 7 bp from a subtidal specimen of *C. claytoniae* from Santa Catalina Island

(Genbank accession MH350895). The Monterey specimens of *C. claytoniae* most likely represent an introduced population because the DNA sequence is identical to specimens from suspected introduced populations of *C. claytoniae* from New Zealand and San Pedro, CA. The Santa Catalina Island specimen, however, which differs by only 1 bp from a specimen of *C. claytoniae* (Genbank accession HQ833786) collected at the type locality on the same date as the type collection (Sangjokam, Goseong, Korea, 12 January 2005), represents a separate introduction.

Examination of specimen images from the specimen database of the University of California's Herbarium (UC), indicates that the Monterey introduction occurred decades ago. Three specimens that resemble *C. claytoniae* were collected in the 1960's from or adjacent to the Monterey marina are: UC1974045, UC1976390, and UC1974044. It is possible that *C. claytoniae* occurs at other coastal harbors in California. However, because this species was misidentified by earlier workers as a larger form of *C. peregrina*, or a variant of *C. sinuosa*, the distribution and abundance of *C. claytoniae* is underestimated.

PYROPIA SUBORBICULATA (Kjellman) J.E. Sutherland, H.G. Choi, M.S. Hwang & W.A. Nelson (BANGIACEAE).—Marin Co., epilithic on large boulders in the upper intertidal, associated with *Pyropia perforata* (J.Agardh) S.C. Lindstrom, Sausalito, 37.856111, -122.478556, thalli sterile and fertile, 26 Dec 2017, *J. R. Hughey s.n.* (UC2050587) and 16 Feb 2018, *J. R. Hughey and M. K. Hughey s.n.* (UC2050590 and UC2050591).

Previous knowledge. *Pyropia suborbiculata* is native to China, Japan, Korea, Phillipines, and Vietnam (type locality: Goto-retto, Nagasaki Prefecture, Japan) (Broom et al. 2002; Vergés et al. 2013), and has been introduced to Australia, Brazil, Iberia, northeastern and northwestern Atlantic, Mediterranean, Mexico, New Zealand, and the northeastern Pacific (Broom et al. 2002; Aguilar-Rosas and Aguilar-Rosas 2003; Klein et al. 2003; Milstein and De Oliveira 2005; Neefus et al. 2008; Hughey 2011; Vergés et al. 2013). Hughey (2011) was the first to report *P. suborbiculata* (as *Porphyra suborbiculata*) from California, where it was found growing attached to barnacles on pilings in Tomales Bay. *Pyropia suborbiculata* has small (up to 4 cm in diameter) ovate to cordate monostromatic blades that are dark brownish red to pink, or bronze and violet (Broom et al. 2002; Aguilar-Rosas and Aguilar-Rosas 2003; Neefus et al. 2008).

Significance. This is the second report of *P. suborbiculata* in California. The structure of gametophytic thalli collected in Sausalito was consistent with previous morpho-anatomical descriptions of this species. Despite the small size of *P. suborbiculata*, its discovery was possible due to the conspicuous dark red blades that were visible from the promenade

and distinct from the larger bladed, greenish black native species *P. perforata*, whose individuals were intermixed with *P. suborbiculata* on the same boulders. The identification of *P. suborbiculata* was confirmed with an *rbcL* gene sequence (GenBank accession MG944247) that was identical to three specimens from Japan (Iwate, Kanagawa, Yamaguchi) and one from Tomales Bay, CA. The DNA methods were the same as those noted above for *Agardhiella subulata*. Given that small recreational boats commonly travel from San Francisco to nearby coastal waters of Bodega, Half Moon, and the Monterey Bays (Zabin et al. 2014), we predict that *P. suborbiculata* will spread to these localities.

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NOTEWORTHY COLLECTION

IDAHO

ISOETES MINIMA (A.A.Eaton) (ISOETACEAE). — Washington Co., Payette National Forest, vicinity of Cuddy Point, 17 air miles W and 3.5 air miles N of Council, ID, approximately 1.5 miles on Forest Road 87 from junction with Forest Road 55, 44.780637°, –116.780851°, elev. 2300 m, in a moist draw with seasonal springs and seeps just SE of dry basalt plateau, associated with *Allium brandegeei* S.Watson and *Balsamorhiza sagittata* (Pursh) Nutt. around edges, also associated with *Lewisia* sp., *Delphinium depauperatum* Nutt., and *Mimulus* sp., 10 July 2017, Harpo Faust 17-105 with David Tank et al. (ID); Adams County, Payette National Forest, Squaw Flat, 6 air miles W and 10 air miles S of McCall, ID, 44.7690319°, –116.249435°, elev. 1908 m, in a seasonally wet meadow dominated by *Camassia quamash* (Pursh) Greene and *Bistorta bistortoides* (Pursh) Small, enclosed by *Pinus contorta* Douglas ex Loudon and *Picea engelmannii* Engelm., 16 June 2018, Megan Ruffley 18-1 with David Tank et al. (ID).

Plant small, < 6 cm, dozens of fertile individuals at each site with both mega- and microsporangia, subula green above ground and white below. Collections were made by the 2017 and 2018 University of Idaho Advanced Field Botany classes based out of the University of Idaho McCall Field Campus in McCall, Idaho, and all collections made on 10 July 2017 and 16 June 2018 in the vicinity of *I. minima* may be accessed via the Consortium of Pacific Northwest Herbaria data portal (CPNWH 2018).

Previous knowledge. *Isoetes minima* is known from fewer than a dozen collections made in the Pacific Northwest across Oregon, Washington, British Columbia, and now Idaho. Each collection site is denoted as a high elevation open meadow or plateau, occasionally on a slight slope, with *I. minima* growing in moist or seasonally wet soil. Based on the existing herbarium collections and the species often associated with *I. minima*, there may be some evidence of a basaltic affiliation, but nothing conclusive.

Isoetes minima was first described from a specimen that was collected in 1889 by Wilhelm N. Suksdorf (Suksdorf 2365), but was not formally described until a decade later by Alvah A. Eaton (1898). The original locality was described as “damp places in prairie near Waverly, Spokane county, Wash.” (Eaton 1898). In a monograph of Isoetaceae, Pfeiffer (1922) reduced *I. minima* to a variety of *I. howellii* Engelm, *I. howellii* var. *minima* (A.A.Eaton) N.Pfeiff. *Isoetes minima* was not included by Hitchcock et al. (1969) in the compilation of their *Isoetes* treatment for the Vascular Plants of the Pacific Northwest, as neither *I. minima* or *I. howellii* var. *minima* was mentioned. *Isoetes minima* was only known from the type specimen until 1985,

almost 100 years after its discovery, when a specimen was collected by Oldrisk Ceska (Ceska 19754) in Colockum Pass, Kititas County, Washington (Taylor et al. 2003). This discovery, along with molecular phylogenetic work (Taylor et al. 2003), resulted in *I. minima* being re-elevated to species status and included in the revised 2nd edition of the Flora of the Pacific Northwest (Giblin et al. 2018). To our knowledge, *I. minima* has only since been discovered in Kititas County (Legler 11766) and Okanogan County, Washington (Beck 201191 and 201145), Wallowa County, Oregon (Thomas 12301), and southeastern British Columbia (Lomer 4391 and 8343, Ceska 30000, and Roemer 96-164).

Significance. These are the first collections of *Isoetes minima* in the state of Idaho, expanding the range of the species from southeastern British Columbia, central to eastern Washington, and northeastern Oregon to include adjacent regions of central Idaho. They also represent the southernmost collections of the species to date. The closest documented locality to these collections is in Wallowa County, Oregon, ~30 km west of Faust 17-105, indicating that these are likely natural occurrences. The lack of other collection records in the state of Idaho could be explained by a number of factors, including the possibility that other populations of this very inconspicuous plant may have been overlooked by collectors, a true rarity of occurrences, and/or that western Idaho may represent the eastern limit of the *I. minima* distribution. Without additional collections, the true conservation status of *I. minima*, which is considered critically imperiled (S1) in Washington and British Columbia and threatened (S3) in Oregon, remains unclear, and its conservation status should continue to be closely examined.

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THE EFFECTS OF LEAF LITTER ON GERMINATION IN THE SERPENTINE ENDEMIC *BOECHERA CONSTANCEI*

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ABSTRACT

Leaf litter accumulation may negatively impact seedling germination by altering key ecosystem properties, such as light availability and soil moisture. The impacts of litter depth may be particularly strong for species in dry environments with low vegetative cover and high light availability. The rare species *Boechera constancei* (Rollins) Al-Shehbaz (Brassicaceae) is endemic to the serpentine outcrops of Plumas and Sierra Counties, where litter accumulation is highly variable and *B. constancei* inhabits a range of litter depths (0–40 mm). In this study, we tested whether increased litter depth impedes germination, and whether populations vary in tolerance to litter depth. Specifically, we quantified the impacts of treatments representing the observed range of litter depth occupied by *B. constancei* on the germination of seed collected from nine populations. Differences in germination were analyzed using a two-way ANOVA. While both source population and litter treatment influenced germination, the interaction term was not significant. Post-hoc Tukey's honest significance tests revealed that germination under both median and high litter treatments was significantly lower than under no litter. The median and high litter treatments did not differ significantly. Differences in germination were observed during dark, cold stratification, suggesting that light availability was not a factor. These results indicate that early life stages of this species may benefit from litter reduction treatments, as both median and high litter treatments reduced germination.

Key Words: *Arabis constancei*, *Boechera constancei*, endemic, germination, leaf litter, management, rare, serpentine.

Plant life histories are shaped by abiotic and biotic influences from the environment. Litter (dead plant material of a small size that is loose on the ground) can facilitate or impede plant growth, by altering temperature, moisture, and light availability (Facelli and Pickett 1991, Baskin and Baskin 1998), and can affect life history stages differently (Muturi et al. 2017). The amount of litter accumulation in an ecosystem determines key properties and functions such as light availability, microbial community composition, and nutrient cycling rates (Sayer 2006). The chemical composition of water and soil is also influenced by litter type and depth. Litter accumulation is typically low on poor nutrient soils, such as serpentine, as plant productivity is lower than on more fertile soils (McNaughton 1968, Gulmon 1992). Therefore an increase in historical litter accumulation may influence the ecology of plants that inhabit these low productivity soils.

Serpentine soils are derived from ultramafic serpentinite and peridotite parent rock material, and comprise over 6000 km² of the state of California (Safford et al. 2005). These soil types are low in essential plant nutrients such as calcium, potassium, phosphorus, and nitrogen, and are rich in heavy metals such as magnesium and iron. In addition, toxic trace elements such as nickel, cobalt, and

chromium are often present in these soils (Safford et al. 2005). This combination results in harsh conditions for plant growth, and few plants are able to tolerate these soil types. These inhospitable soil conditions result in a high degree of plant species endemism (Kruckeberg 1954). Harsh environments like serpentine soils typically have sparse vegetative cover, and a low amount of litter accumulation on the soil surface (Anacker et al. 2012).

In this study we examine the effects of litter on germination in populations of the rare serpentine endemic plant species *Boechera constancei* (Rollins) Al-Shehbaz (Brassicaceae) (Fig. 1). *Boechera constancei* (formerly *Arabis constancei* Rollins) is a strict serpentine endemic, meaning that it is found growing on serpentine soils over 95% of the time (Safford et al. 2005). This rare perennial mustard species is ranked as 1B.1 on the California Rare Plant Rank list, as it is considered “Seriously endangered in California” (CNPS, 2018). *Boechera constancei* populations are restricted to serpentine rocky outcrops in northeastern California (Plumas, Sierra, Lassen, and Nevada Counties). Fire suppression practices in the Sierra Nevada mountain range have caused forests in these areas to accumulate more litter than was historically present (Stephens et al. 2012). Populations of *B. constancei* occur predominately on open



FIG. 1. Photos of *Boechera constancei*. (a) Seedlings in the no litter (control) treatments (photo by Alexander C. Yang), (b) rosette in median litter conditions in Plumas National Forest, and (c) flowering individuals in Plumas National Forest. Photos b and c by Erica J. Case.

rocky outcrops, but can also be found in the nearby mixed conifer forest understory (Case and Harrison 2017). Litter accumulation is highly variable on the serpentine outcrops of Plumas National Forest, and *B. constancei* has been observed to inhabit areas with litter depths ranging from 0–40 mm (Case and Harrison 2017).

Populations of *Boechera* exist in relatively undisturbed environments that have existed for thousands of years (Brunelle et al. 2005). Due to this history, ecologically important genetic polymorphisms have been found to occur across populations of *Boechera* species (Rushworth et al. 2011). In addition, the genus *Boechera* is predominantly self-pollinating, and most species are diploid and reproduce sexually (Schranz et al. 2005, Rushworth et al. 2011). Therefore, we expect to see a high degree of population differentiation with respect to

functional traits in this species, including germination. We predict that we might also see differential responses of populations to abiotic or biotic conditions, such as the presence or depth of litter.

Litter alters the physical and chemical environment on the soil surface, and can impact the demography of plant populations (Facelli and Pickett 1991). Litter presence can inhibit or facilitate seedling emergence, and can have different influences across life history stages in individual plants (Li et al. 2016). Genotype by environment interactions can be strong in some species, and ecotype differentiation in germination among populations has been shown in common garden experiments (Rathcke and Lacey 1985, Facelli and Pickett 1991). Because there is high variability in the litter layer depth between sites where *B. constancei* occurs, and what is known about *Boechera* genetics, it is possible that populations of



FIG. 2. Map of *Boechera constancei* populations in Plumas Co. White diamonds indicate population locations ($n = 9$) (Imagery: Google Earth 2017).

this species may be differently adapted in their tolerance to litter depth.

This study examines whether increased litter depth impedes germination of *B. constancei*, and whether populations of this species vary in their tolerance to litter depth. Specifically, we test the following hypotheses: H1: Increased litter will negatively impact the proportion of germination in *B. constancei*. H2: Populations of *B. constancei* from areas with higher litter accumulation will show greater germination in high litter treatments, due to being locally adapted to these conditions.

METHODS

Nine populations of *Boechera constancei* from Plumas National Forest were selected for this study. Populations were defined as groups of *B. constancei*

individuals that were at least 150 m apart, and up to 40 km from each other (Fig. 2). Sixty populations were visited in total, and seed collection was limited to sites with more than 50 fruiting individuals. Populations used for this study were selected to span the geographical range of species as well as the range of observed litter depths. Seeds were collected from between five and nine individuals per population. No more than 10% of available seed was collected from the population in order to ensure minimal impact to plant populations, and in compliance with our collection permits.

Seed mixes of 240 seeds were then created for each population, and split into 12 replicates of 20 seedlings each. Each replicate was then weighed and assigned to one of three litter depth treatments. Seeds were cleaned in a 20% bleach and 80% deionized (DI) water solution for 20 minutes, and

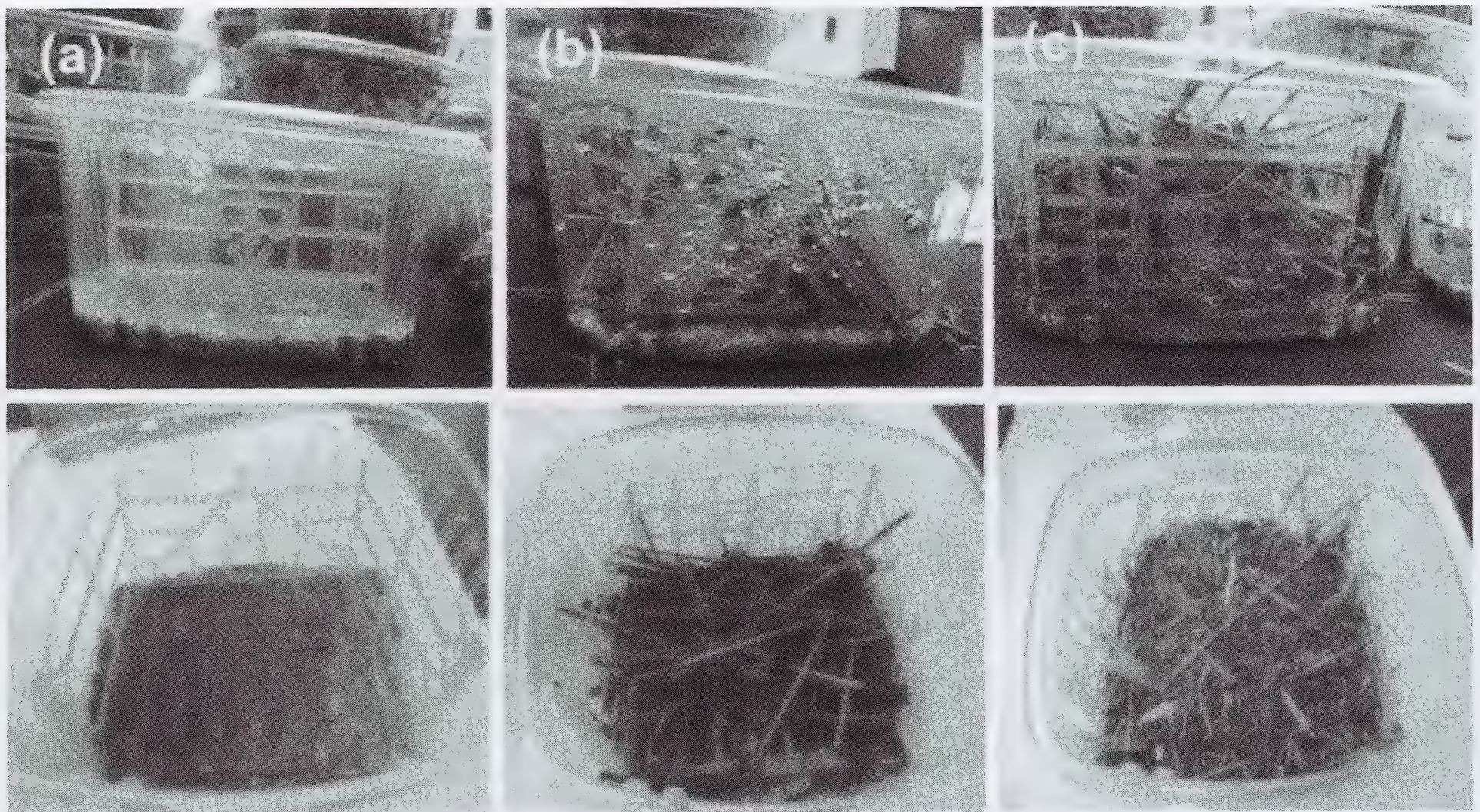


FIG. 3. Litter weight treatments. Shown are two stacked images of each of the following treatments: (a) no litter control, (b) median litter treatment (8 g), and (c) high litter treatment (27 g).

then rinsed with 100% DI water twice before experimental treatments were administered.

Litter treatments were based on field observations of the median (12 mm), high (38 mm) and low (0 mm) litter depths in the areas where *B. constancei* populations occur in Plumas National Forest. To more precisely control the amount of litter in each treatment, litter depths were converted to litter weights by multiplying by average litter density (Case, unpublished data). Specific weights associated with the litter treatments were 27 g for high (38 mm) litter depth observed, 8 g for the average (12 mm) litter depth observed, and 0 g for no litter (control) (Fig. 3). Leaf litter was collected from areas where *B. constancei* populations were found in Plumas National Forest, and consisted mainly of needles and scales fallen from Jeffery pine (*Pinus jeffreyi* Grev. & Balf.), Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*), incense cedar (*Calocedrus decurrens* (Torr.) Florin), sugar pine (*P. lambertiana* Douglas), and Ponderosa pine (*P. ponderosa* Douglas ex. Lawson & C. Lawson). There were four replicates of each litter treatment per population. Treatments were organized in a randomized block design, with 108 total samples, (9 populations x 3 litter treatments x 4 replicates each treatment = 108). This study was conducted at the University of California, Davis.

Previous studies established that *Arabis* spp. (now *Boechera*) seeds require cold stratification at 5°C, for simulation of winter conditions, in order to germinate effectively (Baskin and Baskin 2002; Bloom et al. 1990). We determined via a pilot study on *B. constancei*, that at least 52 days of cold stratification at 5°C produced the highest amount of germination (Olliff-Yang, unpublished data). The replicates were initially put into plastic containers

on wetted germination paper and placed into cold stratification (5°C) in an unlit germination chamber, to simulate winter conditions. The seeds remained in this dark cold stratification for nine weeks (from 8 Decemer 2011–15 February 2012)). On 15 February 2012, the seeds were moved into containers with wet sand. Seeds were placed on top of the sand. Litter treatments were administered at this time. Dried litter was weighed and added to plastic mesh baskets (made from half pint strawberry containers) and placed on top of the seeds (Fig. 3). The mesh baskets were used to remove the litter for watering and to count germination during the study. Control treatments included these baskets as well. Seeds were then put back into dark cold stratification for 23 more days, and then moved into a greenhouse for two weeks. Throughout the experiment, treatments were checked for fungal growth, and when fungal hyphae were observed, seeds were moved into a new treatment container with fresh sand and litter.

Germinated individuals were counted on Day 0, Day 16, and Day 23 of the experiment during dark and wet cold stratification, on Day 27 (after four days in the greenhouse, exposed to light) and on Day 35 (after 12 days in the greenhouse, exposed to light). A seed was considered to have germinated if the radicle had emerged. The development of cotyledons on germinants was also noted. Differences in proportions of germinated seed by litter treatment and population were then analyzed using a two-way ANOVA, after testing to ensure variables met normality assumptions. Post-hoc Tukey's Honest Significant Difference (HSD) tests were done to determine where differences occurred. All analyses were performed in JMP (JMP, Version 9, SAS Institute Inc., Cary, NC).

TABLE 1. EFFECTS OF LEAF LITTER AND SOURCE POPULATION ON GERMINATION. Analysis of variance table of germination by population, litter weight (in grams), and the interaction between population and litter weight. Asterisks indicate significance level: * = $P \leq 0.05$, ** = $P \leq 0.01$, and *** = $P \leq 0.001$. ‘NS’ (not significant) is indicated where $P > 0.05$.

Effect	df	Sum of squares	Mean square	F-ratio	P-value
Population	8	3.092	0.3865	10.874	***
Litter weight (g)	2	0.658	0.6575	18.500	***
Pop x Litter	8	0.356	0.0445	1.253	NS
Residual Error	90	3.199	0.0355		

RESULTS

Litter weight had a significant negative impact on *Boechera constancei* germination ($P < 0.001$, Table 1, Figs. 4 and 5). The germination proportion per replicate of *B. constancei* seeds, as well as the total number of germinants, was significantly reduced in both litter-addition treatments compared to the control treatment (Table 1, Figs. 4 and 5). Post-hoc Tukey’s HSD tests revealed that germination under both 8 g and 27 g of litter were significantly lower than under no litter (Fig. 5, $P < 0.001$). However, both the median and high litter weight had similar effects, and there was no significant difference between the median and high litter treatments ($P = 0.97$, Fig. 5).

Source population had no significant effect on the germination litter relationship. The population that *B. constancei* seeds were collected from did significantly impact germination success, ($P < 0.001$, Table 1 and Fig. 6). However, while both population ($P < 0.001$, Table 1) and litter ($P < 0.001$, Table 1) treatments influenced germination, the interaction term was not significant ($P = 0.278$, Table 1). This reveals that germination success in populations of *B. constancei* does not differ in tolerance to litter weight.

Litter treatments reduced the germination of *B. constancei* in dark cold stratification, suggesting that light availability was not a factor in germination inhibition. Germination and number of total germinants was reduced under litter while *B. constancei*

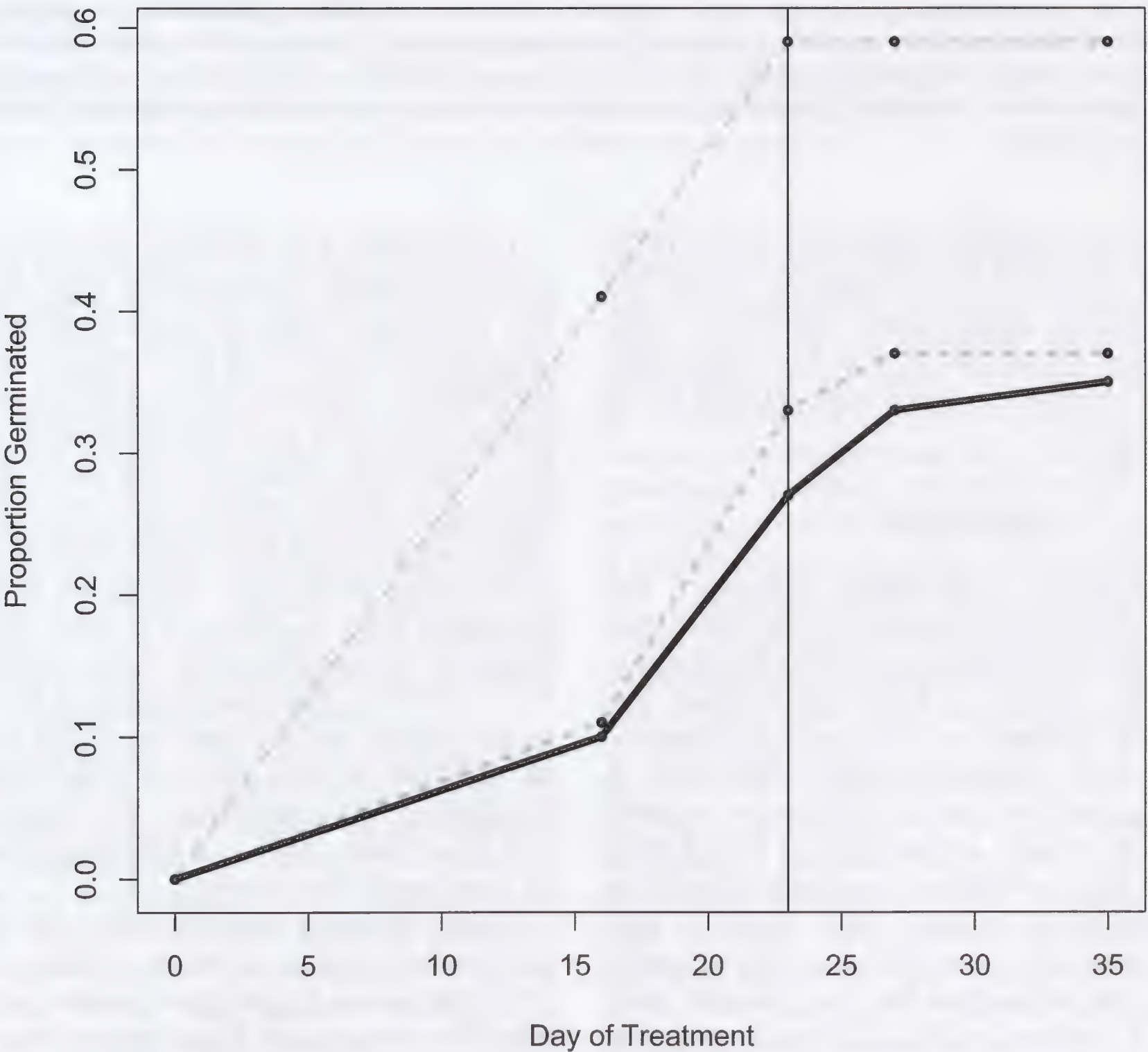


FIG. 4. Mean germination in each treatment over time. Mean proportion of germinated seeds per replicate during the experiment in 0 grams of litter (long dashed light grey line), 8 grams of litter (dotted dark grey line), and 27 grams of litter (solid black line). All replicates were in cold stratification from Day 0 though Day 23 (indicated with a vertical line) of the experiment.

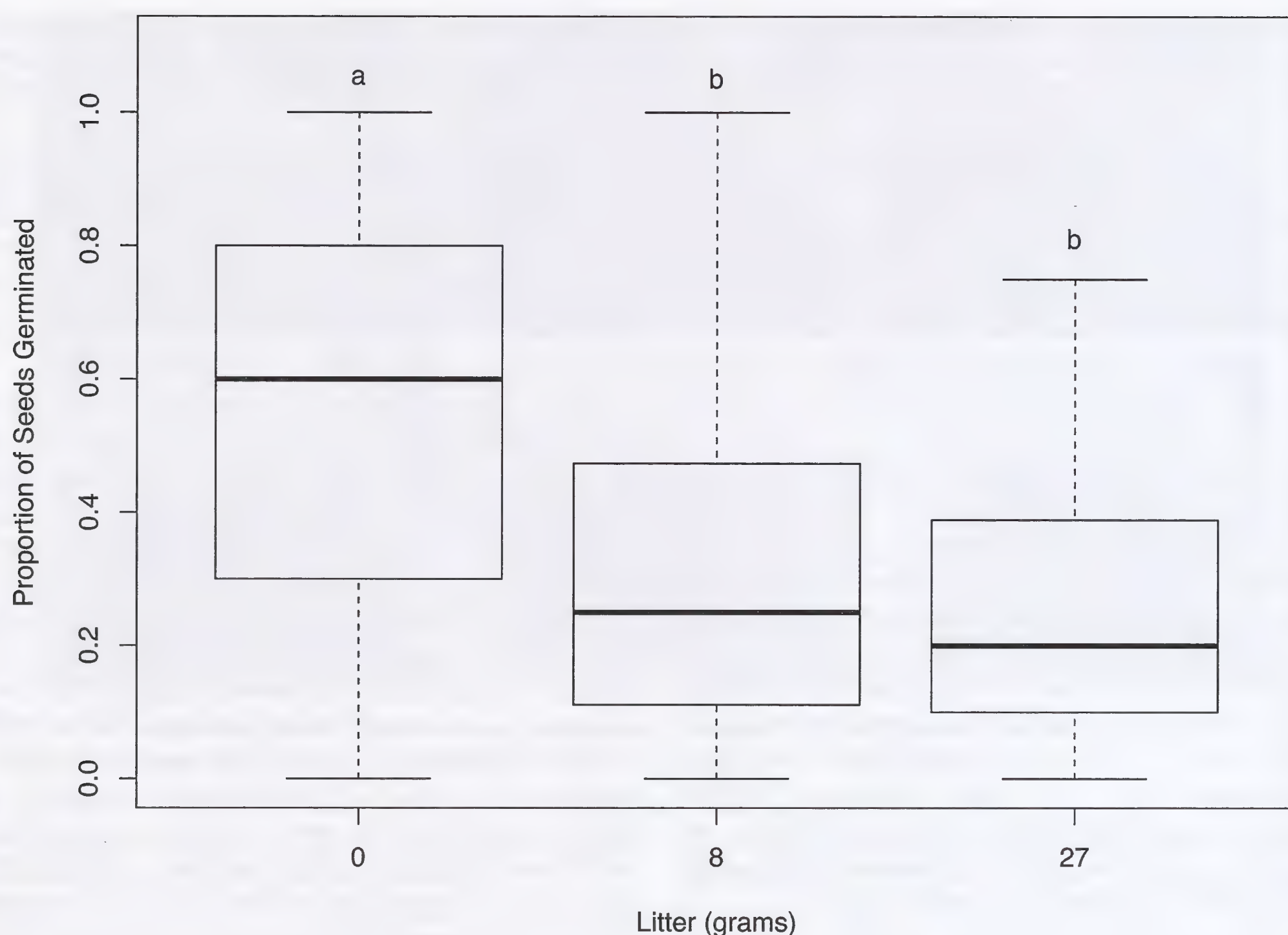


FIG. 5. Proportion of germination by treatment. Proportion of total seedlings germinated per replicate under each treatment, 0 grams of litter (no litter control), 8 grams of litter (median), and 27 grams of litter (high). Midline indicates the median germination, the box indicates the middle 50% of the data, and whiskers show the upper and lower quartiles of the data. Different letters above whiskers indicate significant differences in mean germination proportion between treatments after Bonferroni correction.

seeds were still in cold dark conditions (Days 0–23, Fig. 4). Once moved to lit conditions, there was higher germination under litter treatments than control treatment (Days 24–30, Fig 4). Germination under all litter treatments ceased after 30 days of the experiment (Fig. 4).

DISCUSSION

The germination of *Boechera constancei* was strongly inhibited by the presence of leaf litter, and populations were all similarly impacted (Pop x Litter interaction NS, Table 1). Both the median and maximum litter treatments had lower germination than the control treatment, indicating that *B. constancei* populations could experience reduced germination due to litter accumulation. This finding suggests that litter reduction treatments (such as prescribed burning or manual litter removal) may benefit this species. However, this does not necessarily scale up to the population level, as a recent study reports that *B. constancei* population demography from these and other nearby sites were unaffected by prescribed and natural fires (Case and Harrison 2017). This may indicate demographic differences in the effects of litter on different life stages.

Litter affected *Boechera constancei* germination in dark conditions, revealing that light availability was not the main cause of reduced germination. This suggests that there may be a chemical component to germination inhibition from litter presence. Germination may have been inhibited by tannins or acid from the litter, both of which have been found to delay and inhibit germination (Rice 1979; Kuiters 1989). Once moved to lit conditions, there was higher germination under litter treatments than the control treatment (Fig. 4), but this is most likely due to a chemical component in the litter delaying germination of seeds. Light availability was not a treatment, so we cannot say whether the effects of litter would be different in fully dark or fully light conditions throughout the experiment. We suggest a germination study with dark and light condition treatments to tease apart this question.

Other possible explanations for inhibition of germination include moisture or thermal differences, or differences in fungal growth between the litter and non-litter treatments. Slight temperature or moisture differences may have made a difference between litter and non-litter treatments, as leaf litter buffers temperature (Evans and Young 1970; Facelli and Pickett 1991) and can slow evaporation (Williams et

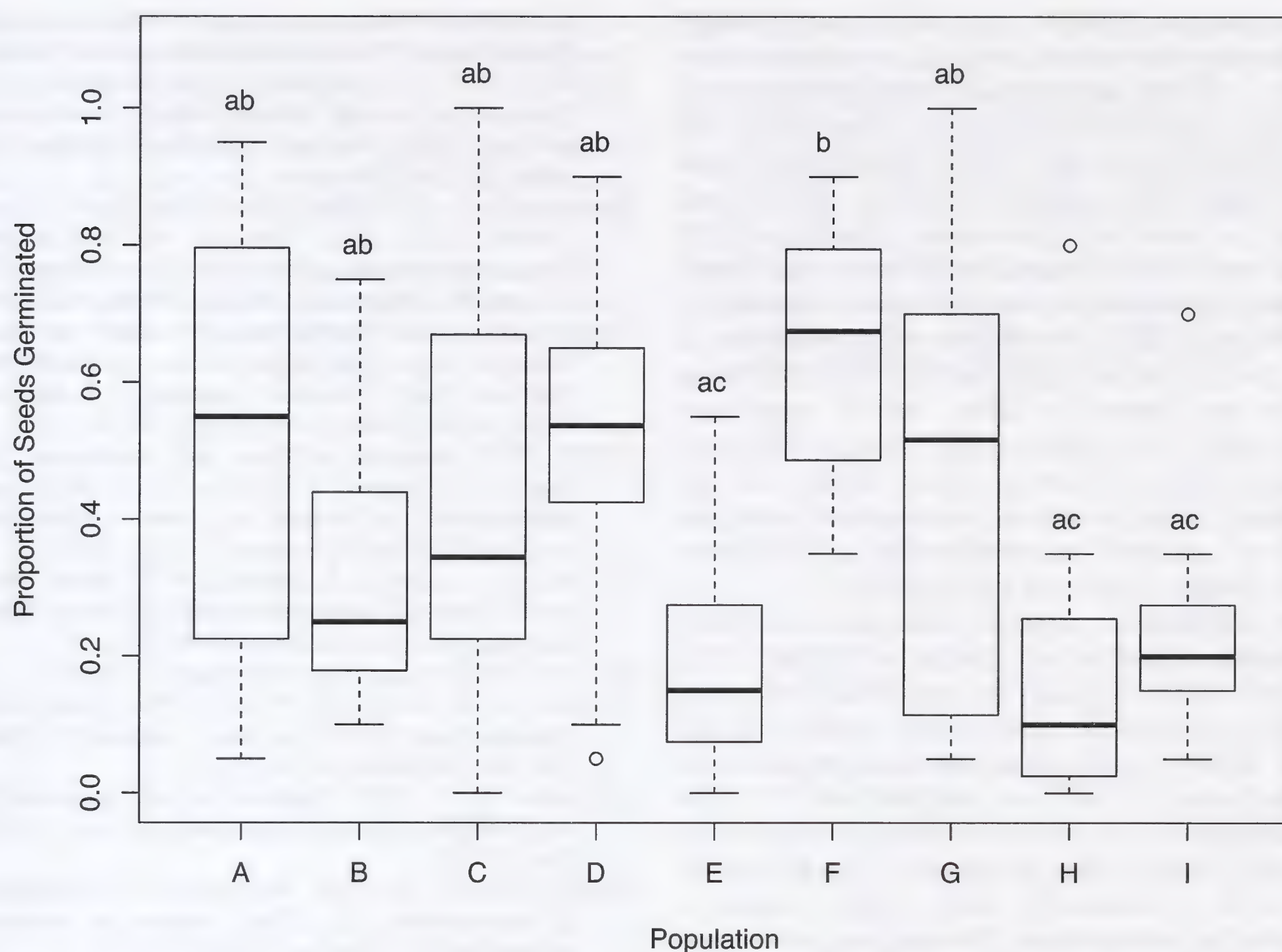


FIG. 6. Germination by population. Mean proportion of seeds germinated compared across populations. Letters correspond to latitude and are arranged in alphabetical order from A, the most southern population (39.91231 N) to I, the most northern population (40.15036 N). Midline indicates the median germination, the box indicates the middle 50% of the data, and whiskers show the upper and lower quartiles of the data. Distinct lettering above the whiskers indicates significant differences in mean germination between populations after Bonferroni correction.

al. 1990). In addition, while we took precautions to reduce fungal growth on the seeds (washing the seeds, drying leaf litter, and moving seeds when fungus was present), litter-addition increased the prevalence of fungal growth in this experiment (Olliff-Yang, unpublished data), which may have contributed to the decrease in germination under the litter treatments. It is unclear whether the same fungal growth under litter occurs in the field. We suggest additional studies manipulating specific abiotic (light, temperature, chemical exudates) and biotic (fungal growth) variables associated with litter accumulation to identify the mechanism(s) by which litter inhibits germination in this species.

There were significant differences among populations in proportion germination (Table 1, Fig. 6), which is consistent with other studies on germination (Baskin and Baskin 1998). We expected to see some difference among populations of *B. constancei*, as this genus is estimated to have lived in relatively undisturbed environments for thousands of years (Brunelle et al. 2005), and because *Boechera* is predominantly self-pollinating (Schranz et al. 2005, Rushworth et al. 2011). However, source population had no significant effect on the germination litter relationship (Pop x Litter interaction NS, Table 1). Therefore, germination differences detected between

populations may be due to local adaptation to other biotic or abiotic factors in the environment. Variation in germination response between populations can occur due to different temperature optima (McArthur et al. 1987). For example, populations of Australian *Cardamine* species vary in their temperature requirement for germination across an elevational gradient (Thurling 1966). Differences in germination at low temperatures can also vary across a species range, as seen in rabbitbrush (*Chrysothamnus nauseosus* (Pursh) G.L.Nesom & G.I.Baird) where germination at low temperatures decreases in populations from cooler higher elevation areas, likely due to differential risks associated with germination in cold temperatures (McArthur et al. 1987). Seeds from different populations have also been observed to vary in sensitivity to soil moisture (*Abies cephalonica* Loudon, Fady 1992), pH and calcium (*Euphorbia thymifolia* Pursh, Ramakrishnan 1965), and salinity (*Dactyloctenium aegyptium* (L.) K.Richt., Okusanya and Sonaike 1991). Differences may also simply be due to variation in seed dormancy proportions between populations (Andersson and Milberg 1998).

Germination is just one stage in the plant life cycle, and therefore inhibition of germination does not directly indicate population decline. When germina-

tion is reduced, other vital life history stages (survival, growth, reproduction) may increase, compensating for the decline, and yielding little or no difference to the overall population growth rate. For example, litter depth may inhibit germination, while at the same time enhance seedling growth and survival, as found in dry woodland species in Africa (Muturi et al. 2017). Opposing influence on different vital rates, also known as demographic compensation (Villellas et al. 2015), may be the reason that litter is shown here to impact germination, while prescribed fire was not shown to affect overall population growth rates in full demographic models (Case and Harrison 2017). However, litter depth did not significantly affect transition rates between plant size classes or variation in reproduction in full demographic models (Case and Harrison 2017).

Litter weight had a negative effect on *B. constancei* germination regardless of population (Table 1, Fig. 5). Litter reduction, such as prescribed fires, may therefore improve the germination success of this species. While overall population demographic rates are not affected by fire (Case and Harrison 2017), the establishment of new populations may be affected, as our results suggest that germination is inhibited by litter depth. Additionally, litter depth should be considered in conservation planning, as germination will be essential for success if management actions include planting or moving seed. Findings from this study have been presented to Plumas National Forest, and results were used in the creation of demographic models for this species (Case and Harrison 2017).

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SURVIVAL OF *PINUS WASHOENSIS* (PINACEAE) FOLLOWING FIRE ON BABBITT PEAK, SIERRA COUNTY, CALIFORNIA

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ABSTRACT

The Cottonwood Fire consumed about 185 square kilometers in the Tahoe and Toiyabe National Forests near Loyalton and Sierraville, CA in 1994. The wildfire reached a population of one of the rarest pines in North America, *Pinus washoensis* H.Mason & Stockw. (Washoe pine), in the Babbitt Peak Research Natural Area (RNA). A field survey and census was conducted in 1996 to find, map, and count living and dead *P. washoensis* individuals, and to assess the effects of the fire on the population. Although the fire reached most of the *P. washoensis* stands, 763 living reproductive individuals were found and less than 5.2% mortality was observed. The fire's intensity declined as it crossed the Bald Mountain ridge and mostly consumed understory vegetation and litter in the *P. washoensis* stands. The fire had the potential to devastate or eliminate this rare population, but instead, a combination of stochastic, vegetative, and topographic controls spared the *P. washoensis* stands and appears to have had beneficial effects for this population. All age classes of *P. washoensis* except seedlings were found in the Bald Mountain Range. The lack of seedlings is surprising because the two years between fire and survey had high precipitation. No sign of hybridization with other yellow pine species was evident. While it is promising that the stands of this rare pine survived the Cottonwood Fire, prospects for the continued persistence of this population are not good until there is a reproductive year that provides many recruits.

Key Words: Babbitt Peak, fire, *Pinus washoensis*, ponderosa pine, survivorship, Washoe pine.

Pinus washoensis H.Mason & Stockw. (Washoe pine), first described by Mason and Stockwell (1945), is one of the most recently recognized pines in the United States and one of the rarest of all North American conifers (Niebling and Conkle 1990). *Pinus washoensis*, a member of Subsection Ponderosae (Critchfield and Little 1966), often is sympatric with its relatives, *P. ponderosa* var. *pacifica* J.R. Haller & Vivrette (Pacific ponderosa pine), *P. ponderosa* Douglas ex Lawson & C. Lawson var. *ponderosa* (typical ponderosa pine), and/or *P. jeffreyi* Greville & Balfour (Jeffrey pine). Critchfield (1984), after 40 years of observations and crossing studies, reported strong reproductive barriers in *P. washoensis*. Hybridization of *P. washoensis* with *P. jeffreyi* and *P. ponderosa* pine occurs, but few viable seeds are produced by interspecific fertilization of *P. washoensis* ovulate cones unless crossed with the Rocky Mountain race of ponderosa pine (*P. ponderosa* var. *scopulorum* Engelm.) (Critchfield 1984). Given the small number of stands and individuals known, *P. washoensis* may be on the verge of extinction, yet its relationship with and possible influence on the commercially important *P. ponderosa* and *P. jeffreyi* underlines the importance of its conservation (Niebling and Conkle 1990).

In spite of the painstaking work of Critchfield, the species has recently been under assault by taxonomists. For various reasons, Lauria (1997) and Brayshaw (1997) dismissed the Washoe pine as a taxon, and considered individuals so-named to be the typical variety of ponderosa pine. However, more

recently Haller and Vivrette (2011) designated a neotype for *Pinus ponderosa*, that solved a nomenclatural crisis in that species and cleared the way to resurrect Washoe pine as a taxon. They went on to declare its taxonomic status as a variety of ponderosa pine, *P. ponderosa* var. *washoensis* (H. Mason & Stockwell) J.R. Haller & Vivrette and cemented that status in the subsequent edition of the Jepson Manual (Haller and Vivrette 2012). After that, three papers looking at the mitochondrial (Potter et al. 2013), nuclear (Potter et al. 2015), and plastid genomes (Willyard et al. 2017) throughout the global distribution of ponderosa pines concluded that Washoe pine was simply a ponderosa pine, and was most closely related to the "North Plateau Race" identified by Critchfield. Willyard and others (2017) also conclude that their results support submerging *P. washoensis* in *P. ponderosa* while splitting the other *P. ponderosa* forms into three previously defined species that currently are considered to be *P. ponderosa*: *P. benthamiana*, *P. brachyptera*, and *P. scopulorum*.

In my experience studying ponderosa pines in the Great Basin for the past 30 years, I believe it is a good taxon and should at least be recognized at the varietal level. The leaves of Washoe pine are distinctive because they are short (8–15 cm) and in fascicles of 3 needles. Ponderosa pines in southern and eastern Nevada mostly have short needles, but when they do, they always have many needles in fascicles of 2. Most ponderosa pines in northwestern and western Nevada have long needles (13–25 cm), in

fascicles of 3 needles, as do those on the west slope of the Sierra Nevada and along the Pacific Coast of California. The cones of Washoe pine are the most distinctive of any *P. ponderosa sensu lato* because of the density of their cone scales, their symmetry, and the dark color of the cone scales' abaxial surface (Kral 1993). Additional morphological differences are well-summarized in Haller and Vivrette (2011). Unique among all the varieties of ponderosa pine are the incurved prickles of Washoe pine cones, making them look and handle like miniature Jeffrey pine cones. Moreover, they are ecologically separated from ponderosa pines when sympatric, with the only reported exception in the Warner Mountains (Rehfeldt 1999).

Pinus washoensis is known to occur in small populations in California mapped by Griffin and Critchfield (1972) from only the Warner Mountains (Modoc County), Bald Mountain Range (Sierra County), Diamond Mountains (Plumas County), South Cascades (Lassen County), and the Carson Range (El Dorado County, Rehfeldt 1999). In Nevada, it occurs in the Carson Range (Washoe and Carson City Counties) and a single tree in the Mosquito Mountains of northern Washoe County (Charlet 1996), all of them on the northwestern boundary of the Great Basin (Griffin and Critchfield 1972; Charlet 1996). In spite of the few small known populations, the species' geographic range spans 310 km north to south, occurs in five National Forests (Toiyabe, Tahoe, Plumas, Lassen, Modoc). It is reasonable to assume that additional stands exist, but remain to be discovered (Lanner 1984).

The Babbitt Peak Research Natural Area (RNA) contains one of the largest populations of *P. washoensis* (Griffin and Critchfield 1972; Critchfield 1984). Critchfield and Allenbaugh (1965) discovered this stand on the Bald Mountain Range ridge, in a narrow belt from Babbitt Peak north at the highest elevations of the range. Talley (1977) found *P. washoensis* to be a component in 113 ha of forest stands in the Bald Mountain Range. Of this, *P. washoensis* was in nearly pure stands or dominant in 77 ha, containing enormous trees up to 1.4 m in diameter and 34 m tall (Talley 1977). The species also occurs in 13 ha of *P. monticola* Dougl. ex. D. Don (western white pine) forests that were being invaded by *Abies concolor* (Gordon & Glend.) Lindl. ex Hildebr. var. *lowiana* (Gordon) Lemmon (California white fir). The remaining 23 ha of *P. washoensis*' distribution on the Bald Mountain Range is accounted for in *P. monticola* — *A. magnifica* A. Murray bis (Sierra red fir) forest stands on east and north slopes and also where the species is emergent in *Cercocarpus ledifolius* Nutt. (curlleaf mountain-mahogany) woodlands on the west slope. The *P. washoensis* population on the Bald Mountain Range was surrounded by scattered pure, even-aged stands of *A. concolor* and *Abies*-dominated *A. concolor* — *P. jeffreyi* forest at the lower elevations of the mountain (Talley 1977). Talley also found poor reproduction in this *P.*

TABLE 1. Annual precipitation from 1986 through 1996 at Sierraville Ranger Station, National Forest, Sierra County, California, compared to the 1981–2010 climate normals. Data from Western Regional Climate Center (2018).

Year	Annual precipitation (inches)
1986	32.41
1987	14.95
1988	13.07
1989	18.99
1990	9.09
1991	19.36
1992	17.79
1993	17.99
1994	16.33
1995	35.64
1996	42.37
1981–2010 Normal	29.73

washoensis population, as he observed only five *P. washoensis* saplings in his thorough survey. Conversely, there are nearly pure stands of *P. monticola*, an uncommon community in the Sierra Nevada, on the RNA.

Talley (1977) found that the *P. washoensis* groves on the Bald Mountain Range are protected from fire from the east by rocky cliffs and forest discontinuities. He predicted that, provided fuel levels were low due to local lightning fires, a wind-driven fire from the west would lack the ability to preheat fuels once it reached the ridge, and this and the ridge could cause the fire to subside to the ground in the large *P. washoensis* stands east of the ridge. However, Talley (1977) suggested that the rise to dominance and growth characteristics of *Abies concolor* in the surrounding forests, and limited Washoe pine reproduction he found in the stands, would put the *P. washoensis* population in the Bald Mountain Range at risk even from rare fires.

By 1994, this danger had increased significantly; 1994 was the eighth consecutive year of below-average precipitation, with half of these years below a standard deviation of the 1981–2010 normals (Western Regional Climate Center 2018) (Table 1). Subsequent insect infestations ensued in the vulnerable *P. jeffreyi* (Jeffrey pine beetle, *Dendroctonus jeffreyi* Hopkins) and in *Abies concolor* (fir engraver, *Scolytus ventralis* LeConte) populations (Steven Seybold, USDA Forest Service, Pacific Southwest Research Station and University of California, Davis, personal communication). Elsewhere in the Tahoe area, up to 90% tree mortality in forest stands was known (Angela Parker, USDA Forest Service, personal communication). Although four catastrophic, stand-replacing fires occurred in the vicinity in the previous 40 years (Tahoe National Forest 2004), no low-level fires had burned in the area for many years, and fuel levels were extremely high (Lance Knoxon, Tahoe National Forest, personal communication). Unusual circumstances in 1994 included a hot summer with no precipitation in June, July, and August. In the 107-year record (1909–2016) of the

Sierraville Ranger Station, no precipitation in these months occurred only five times (1926, 1928, 1981, 1994, and 2008) (Western Regional Climate Center 2018). The result was explosive conditions throughout the Sierra Nevada. In August 1994, the Babbitt Peak RNA was precariously poised for wildfire.

At noon on 16 August 1994, the Cottonwood Fire was ignited by campers (Omer 1994) about 16 km WSW of the Babbitt Peak RNA and burned for 15 days throughout 46,800 acres of the Sierraville Ranger District of the Tahoe National Forest (Tahoe National Forest 2004). At its greatest extent, it was the second largest fire in California in 1994 and used 2739 personnel, 13 helicopters, and 7 air tankers (Henderson and Sion 1994). The Cottonwood Fire was a fuel-driven fire and ladder fuels were sufficient for the fire to spread into tree crowns throughout most of the area. Most suppression activity taken was indirect or flanking, as the extreme behavior and high intensities made direct attacks difficult or impossible. The equipment could not move fast enough after the fire crossed Smithneck Creek to build a line to protect the RNA, and so the fire swept over the Bald Mountain Range. Violent explosions occurred as rolling material ignited fuels below the ridge to the east, and the fire then moved up the ridge from the east. The Cottonwood Fire was finally declared out in December 1994, after burning 185 km² (Lance Knoxon, Tahoe National Forest, written communication).

The purpose of this research in the Bald Mountain Range is threefold: (1) to explore the range to find if any *P. washoensis* survived the Cottonwood Fire, (2) to count and map living and dead individuals of the species, and (3) to determine *P. washoensis* survivorship from the Cottonwood Fire.

MATERIALS AND METHODS

On 23 July 1996, a base camp was established immediately west of the Babbitt Peak Lookout. From this location, the entire area above 2440 m on the Bald Mountain Range north of Babbitt Peak was searched for *P. washoensis* by walking transects for four days along topographic contours sufficiently close to assure the sighting of all trees. The Thommen pocket altimeter (5000 m, Revue Thommen AG, Switzerland) was calibrated twice daily at the Babbitt Peak lookout. The location of each *P. washoensis* individual, whether living or dead, was marked on 7.5' USGS quadrangle topographic maps. Additional *P. washoensis* stands were searched for in Balls Canyon, east of the Bald Mountain ridge.

Discrimination between *P. washoensis*, *P. ponderosa*, and *P. jeffreyi* was accomplished for mature individuals without cones as follows: *P. washoensis* and *P. ponderosa* pine were distinguished from *P. jeffreyi* on the basis of yellow pigment present on the inner bark, while *P. jeffreyi* possesses a maroon inner bark pigment. Long needles (15-22 cm) and large cones (15-26 cm) distinguish *P. jeffreyi* from the

short needles (7-12 cm) and cones (5-9 cm) of *P. washoensis* (Griffin 1993). *Pinus washoensis* and *P. ponderosa* were separated on the basis of the incurved cone scale prickles of the former and the out-curved cone scale prickles of the latter and on the the longer needles of *P. ponderosa* (12-26 cm) compared to the needles of *P. washoensis* (Griffin 1993). Juvenile and sapling-aged trees of *P. ponderosa* and *P. jeffreyi* are difficult to distinguish in the field, but living *P. washoensis* are easily distinguished from both *P. ponderosa* and *P. jeffreyi* by virtue of the short needles of *P. washoensis* compared to long for the other yellow pines. Each mapped tree was visited and identified on the basis of these characteristics. Identification of fire-killed individuals was easily accomplished due to the presence of identifiable cones on and/or under the dead trees.

Botanical collections were made (in triplicate) of all conifer species on the mountain and were submitted to the herbaria at Duke University (DUKE), the University of Nevada at Reno (RENO), and the University of California at Berkeley (UC, Charlet 2296, 2303, 2305). Areas that were burned during the Cottonwood Fire were identified by the presence of recently burned shrubs and trees. These burned areas were followed on foot throughout the study area and were mapped.

The four draft topographic maps were photocopied and spliced. This spliced map was scanned and registered in an ArcMap geodatabase. The registered, spliced map image was set to transparency of 50%. Using this as a template, living and dead individual trees and fire polygons marked on the draft map were digitized into layers within the geodatabase in ArcMap. On the original draft map, there were three areas marked as "100," "100," and "50." The numbers represented the counts of living Washoe pines in those areas. Here, the World Imagery basemap (Arc GIS Map Services, ESRI, Redlands, CA) was used at about 1:2000 scale to mark individual trees within the polygon. The resulting layers were draped over the topographic map set in ArcMap (ESRI, ArcGIS Desktop, Release 10, Redlands, CA) to produce the final copy maps.

RESULTS

The Cottonwood Fire burned most of the north end of the Bald Mountain Range. The greatest destruction occurred in the *Abies concolor* and *Abies concolor* — *P. jeffreyi* forests at lower elevations and the *Cercocarpus* woodlands at high elevations on the western slopes of the range (Fig. 2). Much of the understory burned, but very few trees of any species were killed on the upper eastern slopes near the ridgeline.

I found a 763 *P. washoensis* mature live trees. I found 40 (5.2%) additional Washoe pines that were killed by the Cottonwood Fire. A quarter of the *P. washoensis* mortality occurred within the *Cercocarpus* woodlands on the western slopes of the range, where



FIG. 1. *Pinus washoensis* (center) with *Pinus monticola* (right) on Babbitt Peak, Bald Mountain Range, Sierra County, Nevada.

I counted 12 fire-killed individuals. In pure Washoe pine stands, seven *P. washoensis* individuals were killed by the Cottonwood Fire, 19 were killed in mixed conifer forest communities, and two were killed in *Artemisia tridentata* communities on the Bald Mountain ridge (Table 2).

I found *P. washoensis* in a total of six sections in both the Tahoe and Toiyabe National Forests. The map distance between the most northern and southern *P. washoensis* was 5.0 km, while the areal distance between the most eastern and western *P. washoensis* was 3.0 km (Fig. 2). All age classes of the species were discovered, except for seedlings (individuals germinated that year, with cotyledons). There were hundreds of very large individuals on a south-facing slope on an eastern arm of the main Bald

Mountain ridge. The largest of those measured was 35 m tall with a diameter at breast height of 1.5 m, and may have been the same tree that Talley (1977) measured.

Pinus washoensis occurs on the Bald Mountain Range from 2,300 – 2,975 m and is separated from the nearest *P. ponderosa* population, observed on the west side of Dog Valley, by 4.6 km. *Pinus jeffreyi* was occasionally (19 individuals) associated with *P. washoensis* along the ridge and in *Cercocarpus* woodlands. I found no putative hybridization between *P. washoensis* and *P. jeffreyi* in the Bald Mountain Range, as the species are easily separated here on the basis of needle and cone morphology, each more than twice as long in *P. jeffreyi* than in *P. washoensis*.

DISCUSSION

The Cottonwood Fire had the potential to destroy the *P. washoensis* population on the Bald Mountain Range. Large *Abies concolor* and *A. concolor* — *P. jeffreyi* forests were destroyed (nearly 100% mortality) immediately surrounding Babbitt Peak, but only 5.2% of the *P. washoensis* individuals found were dead.

This study suggests that stochastic events, topographic controls, and lack of ladder fuels in the pure stands of *P. washoensis* led to the survival of the species on Babbitt Peak. For one, the Cottonwood Fire was not wind-driven. Given the explosive, fuel-rich condition of the surrounding forest, winds likely would have killed many more *P. wahoensis* trees. Furthermore, the fire hit the Bald Mountain ridge at 19:00 hr when the temperature was falling, relative humidity was rising, and winds were slowing (Lance Knoxon, Tahoe National Forest, personal communication). The topographic control along the ridge recognized by Talley (1977) likely constrained the fire that night and the fire subsided to the ground instead of taking the crowns of the *P. washoensis* stands. The widely-spaced pure stands lacked ladder fuels capable of bringing the fire to the crowns and instead the fire burned the ground litter and the thick bark at the base of mature trees. Instead of a population-extirpation event, the Cottonwood Fire promoted ecological health in the *P. washoensis* population. The fire removed many of the surrounding dense and diseased stands of *Abies concolor* and *P. jeffreyi*, reduced understory fuel loads throughout the area, and spared nearly all of the *P. washoensis*.

Critchfield and Allenbaugh (1965) noted that *P. washoensis* and *P. ponderosa* are well separated on the Bald Mountain Range and proposed that this population might represent the most genetically pure *P. washoensis* population extant due to the lack of hybridization opportunities here between this species and *P. ponderosa*. Yet, Talley (1977) reported poor reproduction in the Babbitt Peak *P. washoensis* stands. The two years following the Cottonwood Fire had precipitation at the Sierraville Ranger

TABLE 2. *Pinus washoensis* survivorship by plant community structure following the 1994 Cottonwood Fire on the Bald Mountain Range, Sierra County, California.

Community structure	# Alive	# Dead	Survivorship
<i>Pinus washoensis</i> pure stands	539	7	98.7%
Mixed conifer forest	134	19	85.8%
Total forest survivorship	673	26	96.1%
Woodland survivorship	51	12	76.4%
Shrubland survivorship	39	2	95.1%
Total survivorship	763	40	94.8%

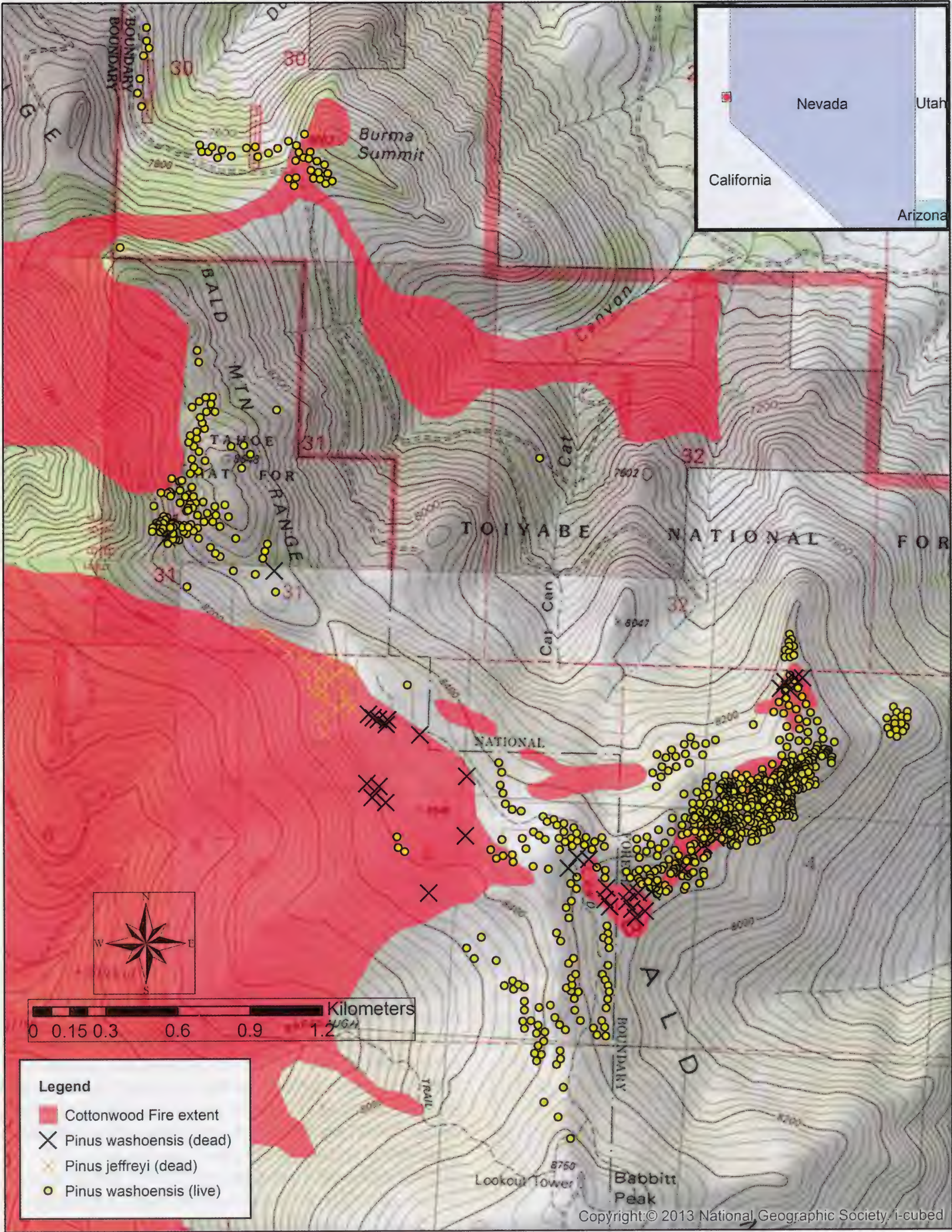


FIG. 2. Cottonwood Fire of 1994 perimeter in the Babbitt Peak area in relation to living and dead *Pinus washoensis* individuals found in the Bald Mountain Range, California, in 1996. Yellow dots indicate living *P. washoensis* individuals, black Xs indicate *P. washoensis* individuals killed by the Cottonwood Fire. Small orange Xs indicate *P. jeffreyi* individuals killed by the Cottonwood Fire in the vicinity of *P. washoensis*.

Station far above the first standard deviation of the annual mean (1995: 90.5 cm, 1996: 107.6 cm, mean = 66.6 cm) (Western Regional Climate Center 2018) (Table 1) and so I expected to see good reproduction in the late summer two years after the fire. However, I observed no yellow pine seedlings anywhere in the study area. Other reproductive events occurred in the 30 years prior to the Cottonwood Fire, but from these, I found only a few more than 20 *P. washoensis* saplings throughout the area in 1996. It may be that the perfect conditions for a reproductive event have not yet occurred, as it has been found in both *P. contorta* Loudon and *P. flexilis* E. James (Millar et al. 2004; Millar et al. 2015) reproduce episodically in the region.

My observations of the distributions of *P. washoensis*, *P. jeffreyi*, and *P. ponderosa* are consistent with those of Critchfield and Allenbaugh (1965). However, even after the Cottonwood Fire, the *P. washoensis* population has more than 750 individuals, many more than the few hundred that Critchfield and Allenbaugh (1965) estimated its size to be. This abundance, combined with the areal separation from *P. ponderosa* and the relative rarity here of *P. jeffreyi*, may partly explain the persistence of this apparently genetically pure population. The lack of intergradation between yellow pines in the Bald Mountain Range and the distinctiveness here of *P. washoensis* supports the argument that there is little or no hybridization occurring in this population. Reproduction in this stand should be monitored in the future.

A question of a reviewer of the submitted version of this manuscript was intriguing – why did I not mention fire scars? I checked my field book and I had made no notes of fire scars on the trees. I returned to the stand in spring 2010 in order to obtain dormant bud samples of thirty individuals of this population to include in a global study of mtDNA haplotypes in ponderosa pine (Potter et al. 2013), and recently examined my notes from that day also: no mention of fire scars on the trees, but I did write that individuals of all age classes were present. Fortunately, I took 240 high resolution photographs of the 30 trees I sampled and their surrounding context on that day, and examined them thoroughly after this question was brought to my attention. None of the Washoe pines had any visible fire scars like those I am accustomed to seeing on ponderosa pines in the Great Basin. It may be that the topographic controls that Tally (1977) first suggested have prevented intense fires from occurring in this stand for centuries.

A new census of the Babbitt Peak Washoe pine population should be conducted, with individuals systematically measured and age classes determined. Wood of individuals should be sampled to find evidence of past fires. The population presents an opportunity to employ multiple approaches to coordinate stand age and population structure with fire history (Harley et al. 2018), both within the

Washoe pine stands and the surrounding ponderosa and Jeffrey pine forests.

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MICROHABITAT CHARACTERISTICS AND IMPACTS OF INVASIVE SPECIES FOR THE TRIPLE-RIBBED MILKVETCH (*ASTRAGALUS TRICARINATUS* [FABACEAE]) IN THE SAN BERNARDINO MOUNTAINS, CALIFORNIA

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ABSTRACT

Astragalus tricarinatus A.Gray is a federally-listed short lived perennial herb endemic to southern California. Its primary habitat is along the ecotone of the Mojave and Colorado Deserts in the San Bernardino and Little San Bernardino Mountains between 390 and 1525 m. Discovery of this species' upland habitat has prompted research into the ecology of populations across its range, as the life history and threats may be different from those of the previously-known wash populations. Our study sought to further document *A. tricarinatus* distribution and abundance within its range, as well as quantify impacts to its growth and reproductive efforts associated with co-occurring native and invasive plant species. We mapped all known localities in our study area on aerial imagery then used this to visually recognize its typical edaphic habitat and identify new potential sites. Where *A. tricarinatus* was found, we established plots in which plant size, the number of reproductive structures, and microhabitat characteristics were recorded. Two new occurrences (defined here as >1 km away from known localities) were recorded within its existing range as well as a previously undocumented locality between two known occurrences containing an estimated 2600 plants. Most plants were found on south and east aspects, and on slopes ranging from 30–45°. We found that increases in invasive cover correlated with a decrease in production of reproductive structures. As a federally-listed species occurring in steep, remote habitats with few threats, these findings suggesting an association between invasive species presence and reduced reproductive structures should be further investigated experimentally, as they affect population dynamics and conservation of this species.

Key Words: *Astragalus tricarinatus*, competition, conservation, endangered species, invasive non-native plants, reproductive effort, rare plants.

The triple-ribbed milkvetch *Astragalus tricarinatus* A. Gray (Fabaceae) is a short lived perennial herb that is endemic to southern California primarily along the ecotone of the Mojave and Colorado Deserts (USFWS 2009; Fraga and Pilapil 2012; Jepson Flora Project 2017). It occurs primarily in the San Bernardino and Little San Bernardino Mountains between 390 and 1525 m, in addition to a disjunct population in the Santa Rosa Mountains. It has also been reported from further east in the Orocochia Mountains by Barneby (1959, 1964), but there is no known specimen for authentication (USFWS 2009; Bell et al. 2017). In 1998, *A. tricarinatus* was listed as endangered by the United States Fish and Wildlife Service based in part on the assumption that it occurred as small, ephemeral populations on benches along desert washes, and canyon bottoms; such occurrences are now known to be waif or deme populations (Barneby 1959; Sanders 1999; USFWS 2009; Fraga et al. 2015). Core habitat is now recognized as topographically rugged, friable soils, often in upper watersheds, and is considered difficult to reach (White 2004; USFWS 2009; Fraga et al. 2015; Bell et al. 2017). These characteristics, together with the difficulty detecting the species visually from a distance, explain why this plant's core habitat had previously escaped detection why this species' population dynamics, reproductive

biology, and ecological relationships are not well understood. Now that presumably stable populations have been identified and mapped, we have the opportunity to glean relevant information about the biology and ecology of the species.

White (2004) first described the occurrence of upland populations of *A. tricarinatus* on exposures of an undescribed white to gray-green colored soil that creates steep, highly eroded slopes with little vegetation cover, often high above the canyon bottom. This observation confirmed what Sanders had predicted in the West Mojave Plan (Sanders 1999; White 2004; Fraga et al. 2015). Observations of individuals found in washes likely originated from those upland, permanent populations (USFWS 2009). For waif populations, threats to the species under the Endangered Species Act (ESA) are not well-documented, but may be related to land-use changes, invasive species, or natural disturbance (Coachella Valley Conservation Commission 2016). Even less, however, is known regarding real threats to upland populations (Sanders 1999; Fraga et al. 2015). Threats to these populations may differ from those known from canyon bottoms in that these are located in remote, steep, and unstable soils, thus it is less clear what factors threaten these populations. Due to their isolation and occurrence largely on protected lands, threats to this species may be limited to the presence

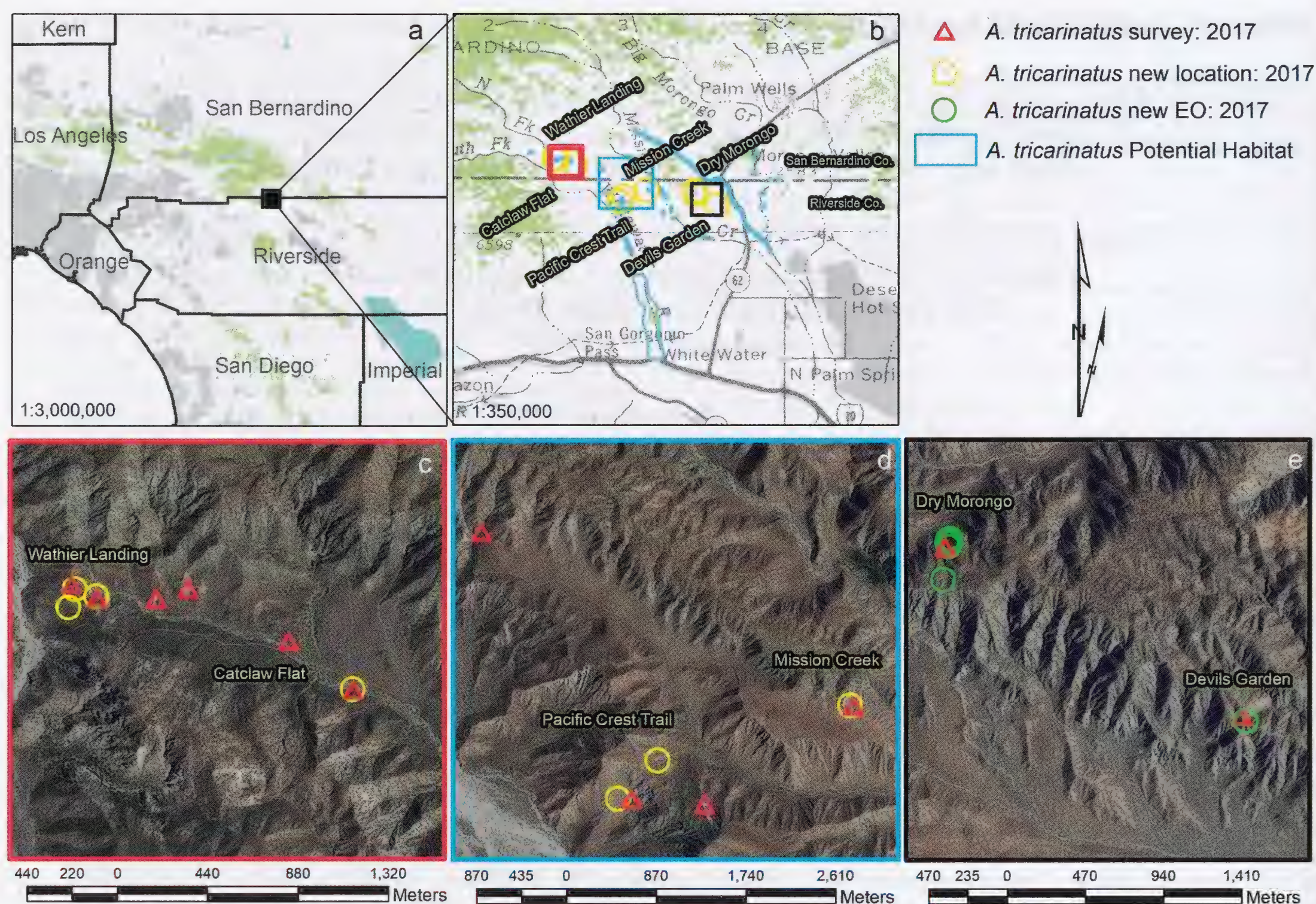


FIG. 1. This multi-panel figure shows: (a) the *Astragalus tricarinatus* study area in southern California (indicated by the black square at the center of the San Bernardino and Riverside County border); (b) the general geographic location in where the surveys were conducted in the southeast portion of the San Bernardino Mountains and; (c–e) specific study sites. Note that the color bordering each map corresponds to the extent indicator rectangles in panel (b). California Natural Diversity Database areas where *A. tricarinatus* might be found are shown in (b) at small scale, and it should be noted that this is not the full extent of its range. The three lower maps (c)–(e) show the areas in true-color aerial imagery (USDA, National Agricultural Imagery Program 2016), displaying the gray-green substrate on which the plant is found. Triangles indicate where surveys were conducted; circles show *A. tricarinatus* locations that were not previously documented, with new Element Occurrences (EO; >one-quarter mile away from known occurrence) in green.

of invasive species and changes in climate (Fraga et al. 2015).

We initiated this study as part of the monitoring of protected species, including *A. tricarinatus*, under the Coachella Valley Multiple Species Habitat Conservation Plan (CVMSHCP), with the aim of ensuring the long-term persistence of self-sustaining populations (Coachella Valley Conservation Commission 2016). Our broad objective was to evaluate threats to persistence of the populations of this species in the San Bernardino Mountains, within the CVMSHCP. Such threats may include human disturbance, invasive species, natural stochastic events, and climate change. Knowledge about the degree to which such threats impact *A. tricarinatus* can then lead to appropriate land management protocols and an update of the listing status of this species (Amsberry and Meinke 2007; Fraga and Pilapil 2012; Fraga et al. 2015). Due to resource-related constraints, we were unable to follow the study protocol laid out by Fraga et al. (2015) or repeat Amsberry and Meinke's (2007) study. Rather we focused on collecting data

directly relevant to the CVMSHCP goals, to explore whether invasive plant species are affecting *A. tricarinatus*, and simultaneously further document the extent of extant populations in the northwestern end of the range, microhabitat characteristics of the upland populations therein, and vegetation associates.

METHODS

Study Area

We concentrated our population assessments for *Astragalus tricarinatus* in the southeast portion of the San Bernardino Mountains (Fig. 1a, b); the eastern portion of the Transverse Range in southern California, which exhibits the typical white to gray-green soil on which *A. tricarinatus* appears to thrive (White 2004; Figs. 1c–e and 2). Known localities of *A. tricarinatus* were mapped and aerial imagery was used to manually search for its typical edaphic habitat. Searches were conducted within in

the Whitewater River, Mission Creek, Dry Morongo and Big Morongo Canyon drainages as they are currently thought to support the largest populations (CNDDDB 2017; Bell et al. 2017; Fig. 1). Although we did survey along canyon bottoms, we focused on localities in upland areas. We did not broaden our survey area into the other regions within *A. tricarinatus*' range where concurrent research was taking place (Joshua Tree National Park by Park biologists and in the Santa Rosa Mountains by Rancho Santa Ana Botanic Garden [RSABG] botanists [Fraga et al. 2015; Bell et al. 2017]).

Data Collection

We surveyed for *Astragalus tricarinatus* within Big Morongo Canyon, Dry Morongo Canyon, Mission Creek and Whitewater River drainages (Fig. 1b) from October 2016 to March 2017. During the winter surveys, we located outcrops of typical soils and looked for evidence of remnant vegetation to inspect later in our spring season *A. tricarinatus* searches; spring season surveys then focused on collecting data on density and reproductive condition (numbers of flowers and fruits). We did not locate any stands to survey within Big Morongo Canyon, or in canyon bottoms. Locations were deemed to be new occurrences if they were greater than one-quarter mile away from other observations of *Astragalus tricarinatus* (*sensu* Bittman 2001) that were listed in the California Native Diversity Database (CNDDDB 2017).

At each population locality we were careful to minimize disturbance. We cleaned all equipment before entering a new area with potential for *A. tricarinatus* to prevent our activities from being a vector for invasive species. We used rappelling equipment for safety, to reduce erosion and for ease of data collection when we had the opportunity to set up an anchor. If plants were on a slope that was too steep and/or unstable to survey without causing unacceptable amounts of disturbance, those populations were not surveyed, but their location was noted.

In each surveyed population, we documented the extent of the area that the population covered, the general slope and aspect, the associated species and total number of *A. tricarinatus* within the stand. The surveys of individual plants were conducted using a protocol we developed prior to surveys (2017) in cooperation with the CVMSHCP Biological Working Group to record specific habitat characteristics of individually monitored *A. tricarinatus*. These occurred with the Dry Morongo, Mission Creek, and Whitewater drainages. To assess the potential for interactions with other plant species, we surveyed 68 random plots, each centered on an *A. tricarinatus* plant within *A. tricarinatus* populations. We recorded the location of each survey plot using a Trimble Juno 3B GPS (Trimble Inc., Sunnyvale) unit and attributes of the plot using a 1 m² plot frame or folding meter stick (Fig. 2). We recorded slope, aspect, all plant



FIG. 2. *Astragalus tricarinatus* plot photo, Little San Bernardino Mountains, CA. Photo credit: James Heintz.

species within the plot and their percent cover (which was allowed to exceed 100% due to overlap but did not within any of our study plots), the above ground volume of the focal *A. tricarinatus* plant (calculated using the formula for an ellipsoid $V = 4/3\pi abc$, $a = 1/2width$, $b = 1/2width$, $c = 1/2height$), and estimated age based on presence of residual rachises (one year or \geq two years). We also measured reproductive effort, which we defined as the number of reproductive structures (combined counts of buds, flowers, and fruits) an individual has produced at the time it was surveyed. We interpreted reproductive effort as the maximum potential fecundity of that plant at that time (in contrast to “reproductive capacity,” from Amsberry and Meinke (2007), which was an estimate of a single season’s seed production, for 2005). Because this study was limited to one year, we were unable to estimate lifetime seed production. We therefore used reproductive effort to evaluate the potential for population persistence and growth at each site and any impacts of plant community correlates. Each plant was photographed close-up and each plot was photographed; close-up photographs often helped confirm field counts for plants with large numbers of reproductive structures (Fig. 2). Plants in each plot were categorized in subsequent analysis as non-native or native species based on information from The Jepson Flora Project (2017), and invasive or non-invasive based on the California Invasive Plant Council Inventory (Cal-IPC 2018).

Our goal in evaluating the data was to better describe the relationships between reproductive effort and site characteristics and to quantify potential threats to *A. tricarinatus*. In statistical analysis, the dependent variable was the “reproductive effort”: number of reproductive structures on each reproducing individual. Data for individual plants were pooled across populations, as reproductive effort did not significantly differ among them. The reproductive effort data were ln-transformed to improve normality when appropriate. The independent variables were the percent cover of all native

TABLE 1. LOCATIONS OF *ASTRAGALUS TRICARINATUS* INDIVIDUALS AND POPULATIONS LOCATED WITHIN THE LITTLE SAN BERNARDINO MOUNTAINS, CA, DURING SURVEYS, ELEVATION, AND APPROXIMATE POPULATION SIZE. Devils Garden Road and Dry Morongo Creek Drainage are new element occurrences and are in bold. With the recording of Wathier Landing North occurrence, all Wathier Landing sites now appear to be one continuous population and are in italics.

Location	Survey date	Approx. elevation (m)	General aspect	Approx. population size
Catclaw Flat Jeep Road	4/18/2017	1075	E	1
Devils Garden Road	3/25/2017	800	S	2
Dry Morongo Creek Drainage	3/25/2017	875–925	W	29
Mission Creek Drainage	3/25/2017	1000	E	20
Pacific Crest Trail Corridor	4/24/2017	900	E	2
<i>Wathier Landing East</i>	<i>4/19/2017</i>	<i>1175</i>	<i>S</i>	<i>4</i>
<i>Wathier Landing South</i>	<i>4/19/2017</i>	<i>1175</i>	<i>S</i>	<i>250</i>
<i>Wathier Landing North</i>	<i>4/19/2017</i>	<i>1200</i>	<i>S</i>	<i>2600</i>

plant species (excluding the focal plant, including any other *A. tricarinatus* individuals present), percent cover of invasive plant species, and the volume of the focal plant. We used linear regressions in R (R, R Core Team, R Foundation for Statistical Computing, Vienna, Austria) to identify correlations between the variables.

RESULTS

New Populations

During preliminary searches, we found two new occurrences, one in the Mission Creek Drainage along Devils Garden utility road (Fig. 1e), and one disjunct occurrence in a drainage leading to Dry Morongo Canyon (Fig. 1e, Table 1). Monitoring started early in the season (late March) for the occurrence on Devils Garden utility road, as one of the two plants was in full fruit by March 25, 2017. The population leading to Dry Morongo Canyon was surveyed in early May as it had a more typical phenological schedule. Over

30 plants were counted on both walls of the canyon within several small drainages.

During our survey of Wathier Landing in White-water Canyon (Fig. 1c), one new locality consisting of a single plant was found south of the trail at Catclaw Flat. We also estimated 2600 individual *A. tricarinatus* in an area between the two previously identified occurrences at Wathier Landing (Table 1). This area, between what was previously recorded as two unique populations, connects the two sites and effectively makes them one large population. This is possibly the largest recorded population of *A. tricarinatus*, comprised of many hundreds of reproductive plants and thousands of seedlings and vegetative plants, with the largest portion of the population in this now-described middle section (Fig. 3).

Demographics, Reproduction, and Associated Topography

We centered our plots on five apparent first-year plants and 62 second-plus year plants. In addition to the first-year plants that were not flowering or fruiting, 18 plants that appeared to be second year or older had no reproductive structures. Of plants that were reproducing, they had an average of 98.6 reproductive structures (min 1, max 756, standard deviation [SD] 134.9; Fig. 4). The reproductive effort showed a significant positive correlation with the estimated plant volume for reproductive plants ($F_{22.26, 1, 42} = 22.26$ on 1 and 42 degrees of freedom [DF], adjusted $R^2 = 0.331$, $P < 0.0001$). The plants surveyed were on slopes that faced all cardinal directions, but most plants were found on south and east aspects, and on slopes generally ranging from 30–45° (mean 39.0, min 11, max 68, SD 12.4; Fig. 5).

Associated Species

Astragalus tricarinatus commonly co-occurred with several native and non-native plant species. The non-natives found, however, were all invasive species. Average total plant cover per plot (including natives and invasives) was low, averaging eight percent (SD 8.2). The most common associates were: *Bromus rubens* L., *Mentzelia albicaulis* (Douglas ex Hook.)



FIG. 3. Approximately 2500 *Astragalus tricarinatus* individuals were found in an area slightly smaller than 5 acres, north of Wathier Landing in the San Bernardino Mountains, CA. Photographed are 34 × 2+ year-old individuals along with numerous seedlings growing in typical gray-green substrate. Photo credit: James Heintz.

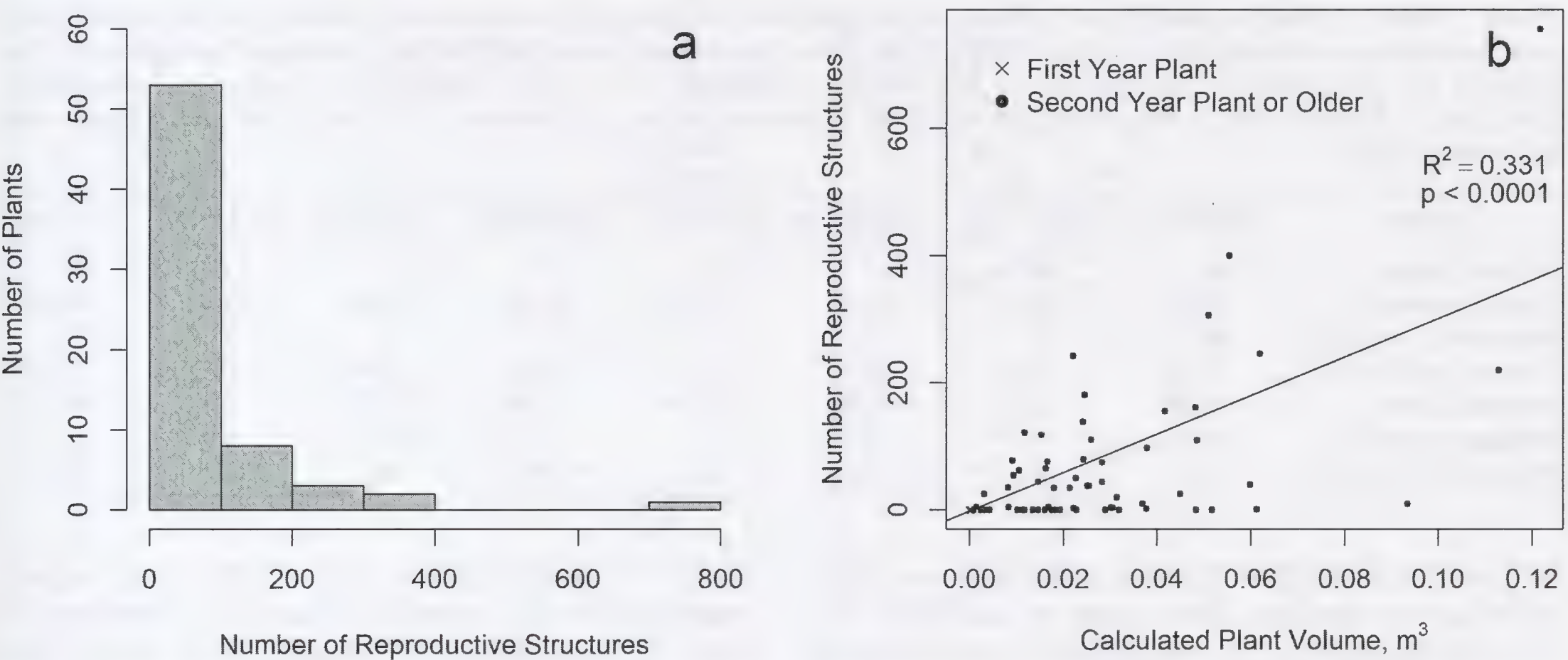


FIG. 4. Reproductive effort of *Astragalus tricarinatus* surveyed in the San Bernardino Mountains, CA, in terms of the number of individuals (a) and the relationship with estimated plant volume (b). The fitted line and correlation shown in (b) includes only reproductive second year individuals (n = 44).

Douglas ex Torr. & A. Gray, *Chaenactis glabriuscula* DC., *Gutierrezia microcephala* (DC.) A. Gray, *Erodium cicutarium* (L.) L'Hér. ex Aiton, *Chaenactis fremontii* A. Gray, *Salvia columbariae* Benth., and *Stephanomeria pauciflora* (Torrey) Nelson (Table 2).

Invasive plant species were found in a majority of the study plots (72%). In particular the annual grass *B. rubens* was the most common plant species we observed growing in and around *A. tricarinatus* populations, occurring in 63% of the sample plots along with invasive annual forb *E. cicutarium* growing in 22% of the plots. *Schismus barbatus*, (L.) Thell., the other invasive species found in six study plots, was only present at >1% cover in one plot and is therefore not summarized individually here. For all plots, average percent cover of all

invasives combined was 1.88% (SD 4.1), and native cover averaged 11.43% (SD 9.3) (Table 3). While there was not a particularly high percent cover of plants, *A. tricarinatus* reproductive effort was significantly lower in the presence of invasive species (especially when invasive cover was $\geq 5\%$) (adjusted $R^2 = 0.306$, F 19.96 on 1 and 42 DF, $P < 0.0001$) (Fig. 6a). The relationship between native cover and *A. tricarinatus* reproductive effort was non-significant (adjusted $R^2 = -0.0172$, F 0.2741 on 1 and 42 DF, $P = 0.603$) (Fig. 6b).

DISCUSSION

With invasive grasses continuing threaten native species in California's deserts, more research needs to

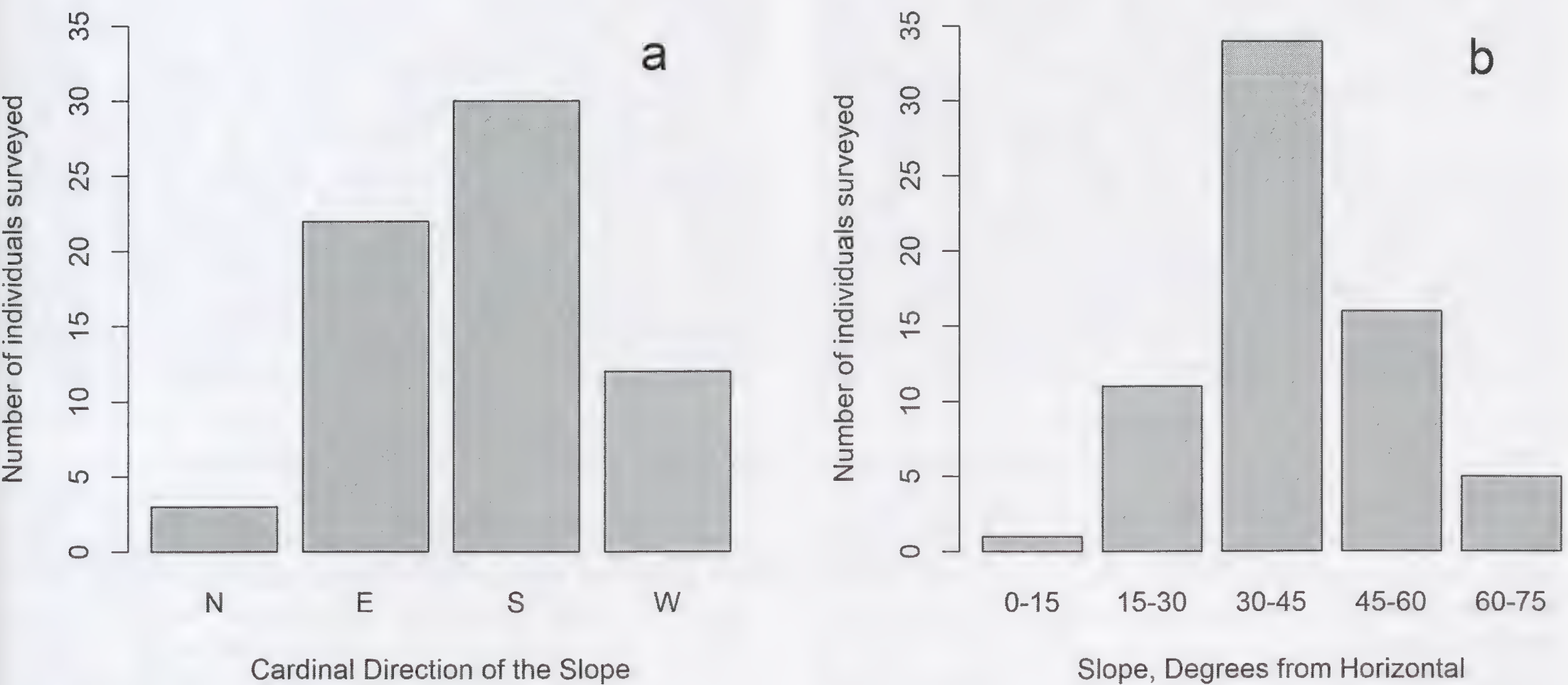


FIG. 5. Cardinal direction (a) and slope (b) for *Astragalus tricarinatus* plants (n = 67) in surveyed plots in the San Bernardino Mountains, CA.

TABLE 2. MOST COMMON ASSOCIATED SPECIES FOUND WITHIN *ASTRAGALUS TRICARINATUS* SAMPLE PLOTS ACROSS ALL POPULATIONS STUDIED: *BROMUS RUBENS* (BRORUB), *MENTZELIA ALBICAULIS* (MENALB), *CHAENACTIS GLABRIUSCULA* (CHAGLA), *GUTIERREZIA MICROCEPHALA* (GUTMIC), *ERODIUM CICUTARIUM* (EROCIC), *CHAENACTIS FREMONTII* (CHAFRE), *SALVIA COLUMBARIA* (SALCOL), AND *STEPHANOMERIA PAUCIFLORA* (STPAU). Invasive species are indicated in bold.

Species	BRORUB	MENALB	CHAGLA	GUTMIC	EROCIC	CHAFRE	SALCOL	STPAU
N plots present	42	20	19	19	15	15	13	10
Percent present	62.69	29.85	28.36	28.361	22.39	22.39	19.40	14.93
Maximum cover (%)	20	3	1	9	1	5	1	3
Average cover (%)	2.73	0.52	0.29	1.73	0.22	0.49	0.31	1.04
Standard error	0.76	0.16	0.09	0.59	0.08	0.33	0.11	0.32
Median cover (%)	0.1	0.1	0.1	1	0.1	0.1	0.1	1
Minimum cover (%)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

be done clarifying how they are affecting rare plants in the region (Brooks 2000; Fraga et al. 2015). Dry nitrogen deposition from smog-laden winds from the Los Angeles Basin to the west have created conditions that foster high invasive species densities, especially for *Bromus rubens*, across this region (Fenn et al. 2003; Ochoa-Hueso et al. 2011). Here, despite these potential threats and five years of drought (conditions that are predicted to increase as climate continues to create warmer-drier conditions in the arid southwest [Diffenbaugh et al. 2008; Gao et al. 2012; Cook et al. 2015]), *Astragalus tricarinatus* populations were found to contain plants in abundance. Thus, this study sought to determine whether the presence of invasives poses a legitimate threat to reproductive effort of this rare and endangered plant.

Apart from the occurrence near Mission Creek Preserve, *A. tricarinatus* grows largely outside of heavy invasive plant influence. However, our data indicate that even at the low densities of non-native plants we found associated with *A. tricarinatus* (all found were known to be invasive [Cal-IPC 2018]), there was a negative correlation between invasive cover and reproductive effort. We consider this finding preliminary, however, due to the very low cover of invasive species, and the small measured decline (−0.22 estimated per-unit reduction in reproductive effort). This finding needs to be further investigated. Our surveys occurred from fall 2016 to spring 2017 during which the rainfall exceeded the long-term mean (Whitewater Station, Western Regional Climate Center, www.wrcc.dri.edu). Therefore, we expect invasive species densities were higher during this sampling period than they would be in years characterized by a more typical climate regime than 2016-2017. Consequently, it is not clear if this

threat is restricted to rare years with higher precipitation. An associated question is whether the level *A. tricarinatus*’ of reproductive effort and seedling recruitment we documented is also restricted to these infrequent comparatively wet years, or if the timing of reproduction was affected by increased precipitation. It could be that reproduction shifts phenologically in response to drier conditions—we did note an earlier reproductive phenology in the new Devils Garden occurrence, where one of the two plants were in full fruit by late March, likely due to its exposed position on the south facing shoulder of the road bed (LaDoux, Joshua Tree National Park, personal communication). We found an average of 99 reproductive structures on reproductive plants, compared with a mean of 228 pods found previously (Amsberry and Meinke 2007). This, however, is not likely to be a real decline because we found high intra-annual inter-site variation (over the 13 general sites surveyed this year, mean numbers of reproductive structures ranged from 3–292).

Invasive species can negatively impact or interfere with native species including rare plants (Huenneke and Thompson 1995; Brooks 2000; Moroney et al. 2011). We have shown that invasive species may negatively influence *A. tricarinatus* by lowering the number of reproductive structures and possibly indirectly by interfering with associated natives (Huenneke and Thompson 1995; Hobbs and Mooney 1997; Brooks 2000; Moroney et al. 2011) through investigating the correlations between co-occurring plants and *A. tricarinatus* reproductive effort. In contrast, we found no effect of competition between native species and *A. tricarinatus*, even though natives were present at a generally higher cover than invasive species. Experimental weeding around a

TABLE 3. COVER OF NATIVE AND INVASIVE PLANTS WITHIN *ASTRAGALUS TRICARINATUS* STUDY PLOTS.

	Total native species cover (including the focal <i>A. tricarinatus</i>)	Associated species cover (excluding the focal <i>A. tricarinatus</i>)	Invasive species cover
Average cover (%)	11.43	5.39	1.88
Median Cover (%)	9.3	3.1	0.1
Sample size (n)	67	67	67
Standard deviation	9.27	7.39	4.13
Standard error	1.13	0.90	0.51

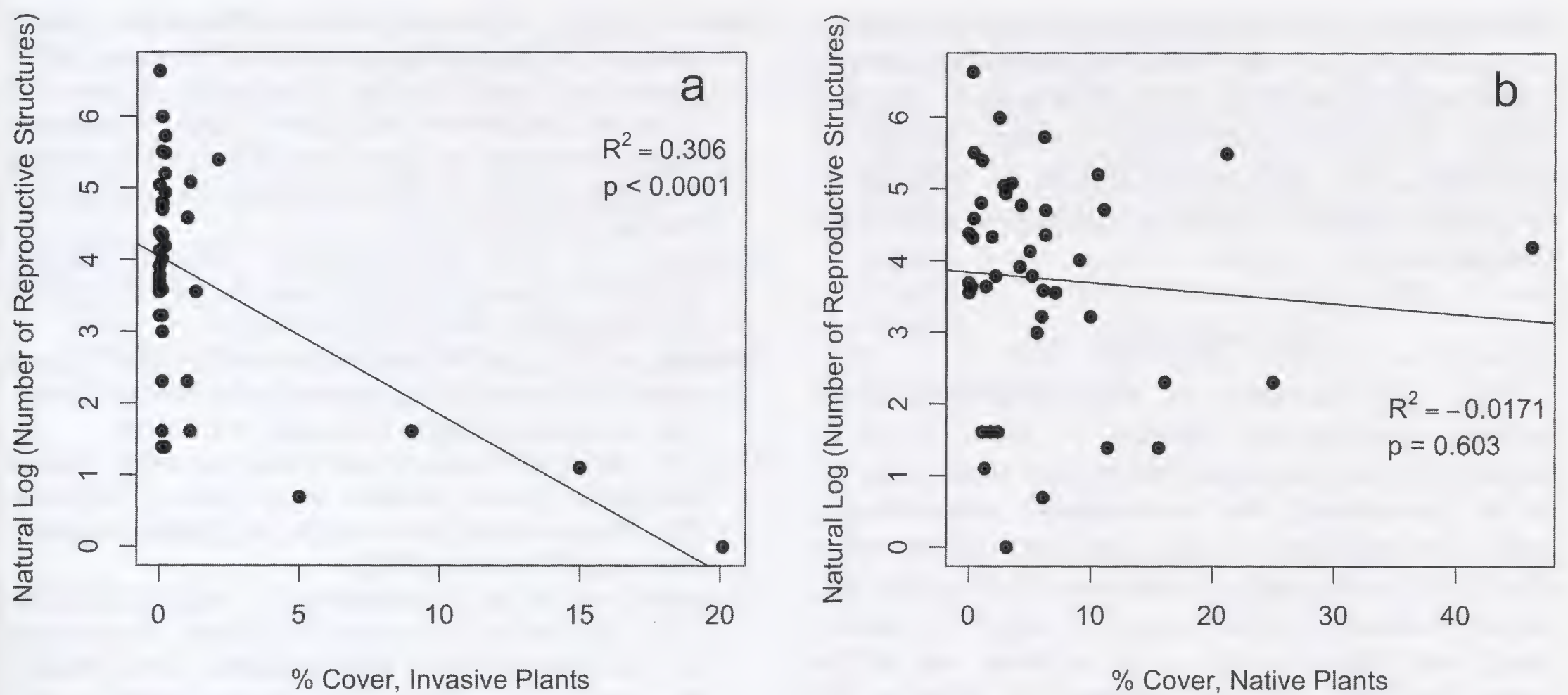


FIG. 6. Relationship between reproductive effort (ln-transformed) of *Astragalus tricarinatus* plants ($n = 44$) and the percent cover of (a) invasive plants (*Bromus rubens* and *Erodium cicutarium*) and (b) native plants (excluding the focal plant) within the study plots in the San Bernardino and Little San Bernardino Mountains, CA.

portion of *A. tricarinatus* plants, while leaving others an un-weeded control, could clarify a causal relationship between the two, although it should be undertaken with extreme caution considering the highly sensitive (steep, erodible) areas concerned. We would recommend further study and analysis to determine which of the invasive species concerned has an ecologically-relevant impact on *A. tricarinatus*, as the three species present, *Erodium cicutarium*, *Bromus rubens*, and *Schismus barbatus* may not all interfere with *A. tricarinatus* or be of equal consequence.

Relevant to conservation efforts and listing criteria, in terms of other threats to the species, we did not document any other factors that would likely have negative impacts on the species. Given this plant's preferred habitat, we did not encounter any threats posed by development, Off Highway Vehicle (OHV) travel, or foot traffic during our surveys. The plants typically occur in areas and on slopes that are not conducive to human travel, whether on foot or vehicle, characteristics that have impeded regular efforts to survey and monitor populations (Fraga et al. 2015). Except for one population just west of the Pacific Crest Trail and east of Catclaw Flats, which had an old pipeline that did not appear to be in current operation and the occurrence of two plants on the shoulder of Devils Garden Road, none of the locations we visited had any impending development or major maintenance threats. However, many of these occurrences were located near to or within a trail corridor, which would expose them to greater invasion risk than populations that are secluded and have minimal foot traffic. The new Dry Morongo upland occurrence (Fig. 1e) may be the source population for waifs and demes found in Dry Morongo Canyon (Bell, Rancho Santa Ana Botanical Gardens, personal communication), a lowland

site that may have a higher cover of invasive plants than the upland sites surveyed in our study.

While surveying the population of *A. tricarinatus* east of Wathier Landing, the author J. Heintz observed scat which appeared to be mammalian with several opened seed pods nearby. It appeared as if the animal had collected the pods to eat while sitting on a high perch which is consistent with the behavior of a fox or other small- to medium-sized mammal. This observation could be indicative of a possible seed disperser; however, the examination of the scat did not reveal anything recognizable as *A. tricarinatus* seeds or seed pods (although any fleshy seeds were likely digested and therefore not detectable in scat [Mark Fisher, University of California, Boyd Deep Canyon Desert Research Center, personal communication 2017]). This does not necessarily mean that they were not consumed, but that the animal may not have been eating a consistent diet of *A. tricarinatus* seed pods prior to defecation, or that they become unrecognizable from digestion.

This study was meant to begin evaluating a threat to the species, as well as to document life history, and associated topography and associated plant species. Future surveys should follow on these two studies to further document longevity, demography, and other important aspects of this species' biology, as suggested by others (Sanders 1999; Fraga et al. 2015), both in upland populations and in canyon bottom waif populations.

Here we have documented several new pieces of a puzzle that are important to the conservation of *A. tricarinatus*. First, we have documented or more closely mapped several populations. Second, we have found a relationship between *A. tricarinatus* reproduction and the abundance of invasive species, which is suggestive of a competitive relationship, negatively impacting *A. tricarinatus*. Lastly, the natural history

observation of a possible dispersal vector (mammal) is encouraging, as very little is known about how these plants maintain their position high on exposed slopes. Together with other studies previously mentioned, this information should be valuable to range-wide as well as local conservation efforts for this species.

Recommendations

With the discovery of this species' preferred habitat, opportunities abound to learn about its biology, as very basic natural history traits have yet to be discovered. We recommend conducting a pollination study across the vegetation alliance types inhabited by *Astragalus tricarinatus* to document and collect pollinators from across its range. To understand seed dispersal for *A. tricarinatus*, we recommend installing wildlife cameras to monitor the various populations to determine if mammals or other vertebrates are collecting and moving the seeds. Pertinent to the substrate on which the species occurs, since it seems to be edaphically restricted, it is important to understand to composition of the observed white to gray-green soil as well as other substrates it is found growing on, particularly the waifs. Also, genetic studies could be invaluable in elucidating how the upland and canyon bottom populations are related, and how each contribute to regional gene flow and habitat occupancy, and whether waif populations are subject to similar or separate threats to persistence. Lastly, this study should be repeated and augmented to include the JTNP protocol in order to form a more robust data set with those neighboring surveys.

ACKNOWLEDGMENTS

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CHORIZANTHE APHANANTHA (POLYGONACEAE: ERIOGONOIDEAE),
A NEW SERPENTINE-ENDEMIC SPECIES FROM THE SAN LUIS RANGE OF
WESTERN SAN LUIS OBISPO COUNTY, CALIFORNIA

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ABSTRACT

Chorizanthe aphanantha K.M.Nelson & D.J.Keil is described as a new species from serpentine soils in the Irish Hills portion of the San Luis Range of central-western San Luis Obispo County. It occurs in portions of San Luis Obispo's Irish Hills Natural Reserve, where it grows with several other rare, serpentine-endemic taxa. It is distinguished from *C. breweri* S.Watson, with which it co-occurs and may be confused (particularly as it ages), by its compact, rather than sprawling habit; the green rather than red color of its herbage at the time of flowering; its much smaller, white, monomorphic perianth lobes, rather than white to pink, dimorphic lobes; bracts with straight, ascending, rather than recurved spine tips; smaller and smooth rather than transversely corrugated mature involucre; and basal leaf blades that are glabrous to minutely strigose, thin, and ovate to elliptic (occasionally mucronate) with obscure veins, rather than thick, leathery, reniform, and apically notched to \pm obcordate with pronounced veins. It differs from the recently described *C. minutiflora* R.Morgan, Steyer, & Reveal by its sparser and shorter pubescence, entire versus erose perianth lobes, nine rather than three stamens, ovate to elliptic rather than oblanceolate basal leaves, and flat rather than undulating leaf margins. It is distinguished from the morphologically similar *C. procumbens* Nutt. by its ovate to elliptic rather than oblong to oblanceolate leaf blades, its sparser and shorter pubescence, the presence of a narrow scarious margin on the involucral teeth, smaller flowers, and distinct rather than basally connate filaments. A key is provided that distinguishes *C. aphanantha* from other *Chorizante* species in the San Luis Range. It is currently known to grow in serpentine scrub and chaparral at elevations from 100 to 370 meters.

Key Words: *Chorizante*, Eriogonoideae, Polygonaceae, *Pungentes*, San Luis Obispo, serpentine-endemic.

Chorizante aphanantha was discovered during a spring 2017 botanical survey of the Waddell Ranch, a parcel in the northeastern part of the Irish Hills, which border the southwestern edge of the City of San Luis Obispo (Fig. 1). The Irish Hills form the western half of the San Luis Range, a small northwest- to southeast-trending range located in central-western San Luis Obispo County, California. The Waddell Ranch property was acquired by the City of San Luis Obispo in January 2017 as an extension of the existing Irish Hills Natural Reserve. The ranch was in private ownership prior to the City's acquisition, and had been maintained in a relatively natural state with limited anthropogenic disturbance. Prior to adding the Waddell Ranch property to the Irish Hills Natural Reserve and opening it to the public for conservation and recreational purposes, the City had the new parcel surveyed for biological resources, in order to inform necessary updates to the existing *Irish Hills Natural Reserve Conservation Plan Update* (City of San Luis Obispo 2011). Prior to these surveys (Nelson 2017),

the Waddell Ranch had never been assessed for botanical resources.

The new species was initially recognized as unique from anything else encountered on the property or in the region based on its minute white flowers, bright greenish involucre, and delayed flowering time compared to *C. breweri* S.Watson, with which it grows in close proximity (Figs. 2, 3). The plant was initially collected by the first author and keyed to *C. procumbens* Nutt. However, further inspection revealed novel characters inconsistent with those of *C. procumbens*. Consultation with the second author confirmed the identification of an undescribed species, resembling, in some aspects, *C. procumbens* and *C. minutiflora* R.Morgan, Steyer & Reveal, neither of which is known to occur in San Luis Obispo County.

TAXONOMIC TREATMENT

Chorizante aphanantha K.M.Nelson & D.J.Keil, sp. nov. (Figs. 2–6) — TYPE: USA, CALIFORNIA, San Luis Obispo Co., San Luis Range, Irish Hills, Irish Hills Natural Reserve, City of

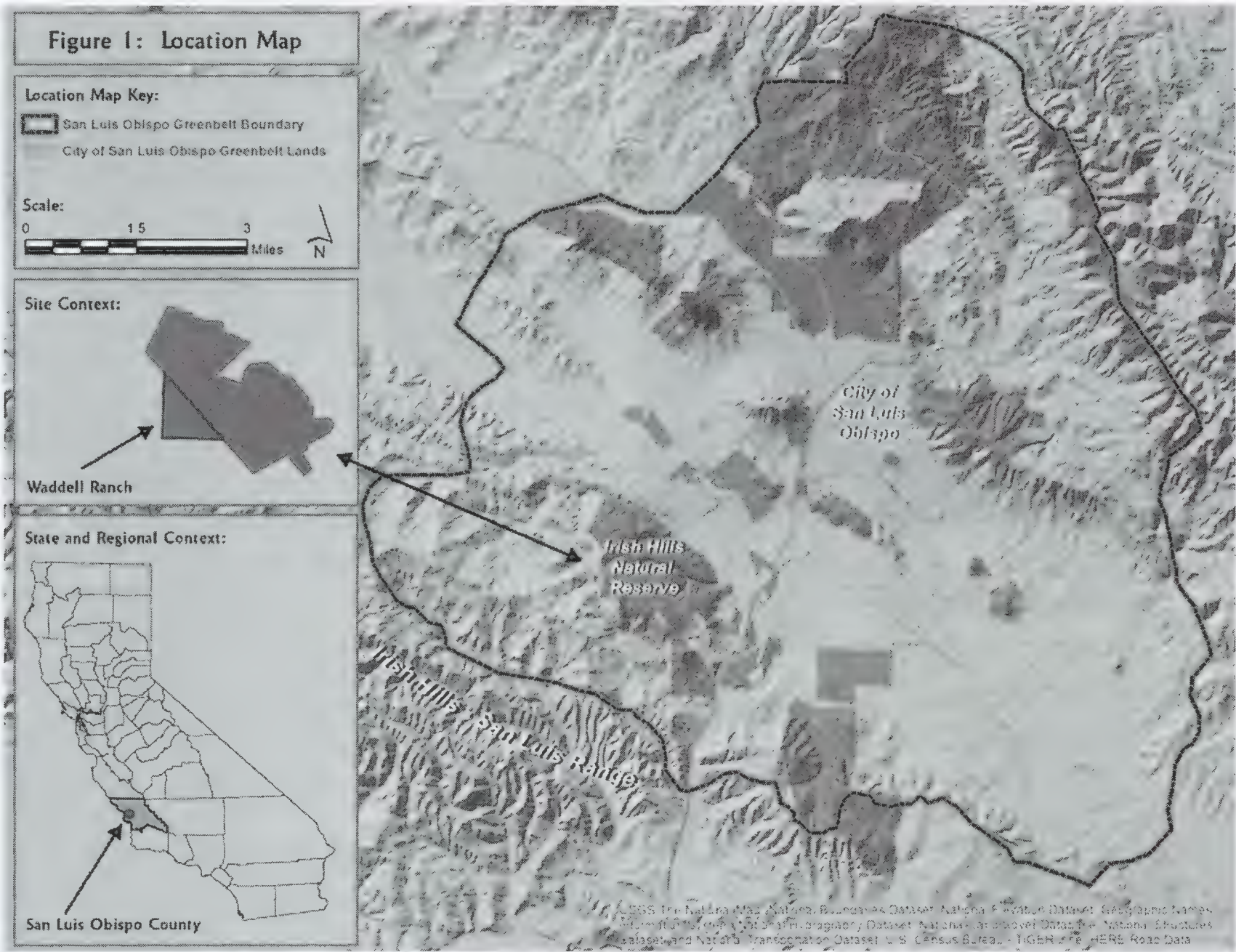


FIG. 1. Regional vicinity map of the Waddell Ranch property and Irish Hills Natural Reserve. Cartography by Robert A. Hill.

San Luis Obispo, Waddell Ranch acquisition; 35.252545°, -120.719224°, 305–312 m, upper south-facing slope and crest of a serpentine hill above From Creek, in openings among shrubs in scrub and chaparral, rocky to gravelly soils and outcrops, 17 June 2017, *Kristen M. Nelson 29 with David J. Keil, Matt Ritter, Jenn Yost, Brooke Langle, Robert Hill, and Kyle Nessen* (holotype: OBI-83736; isotype: UC).

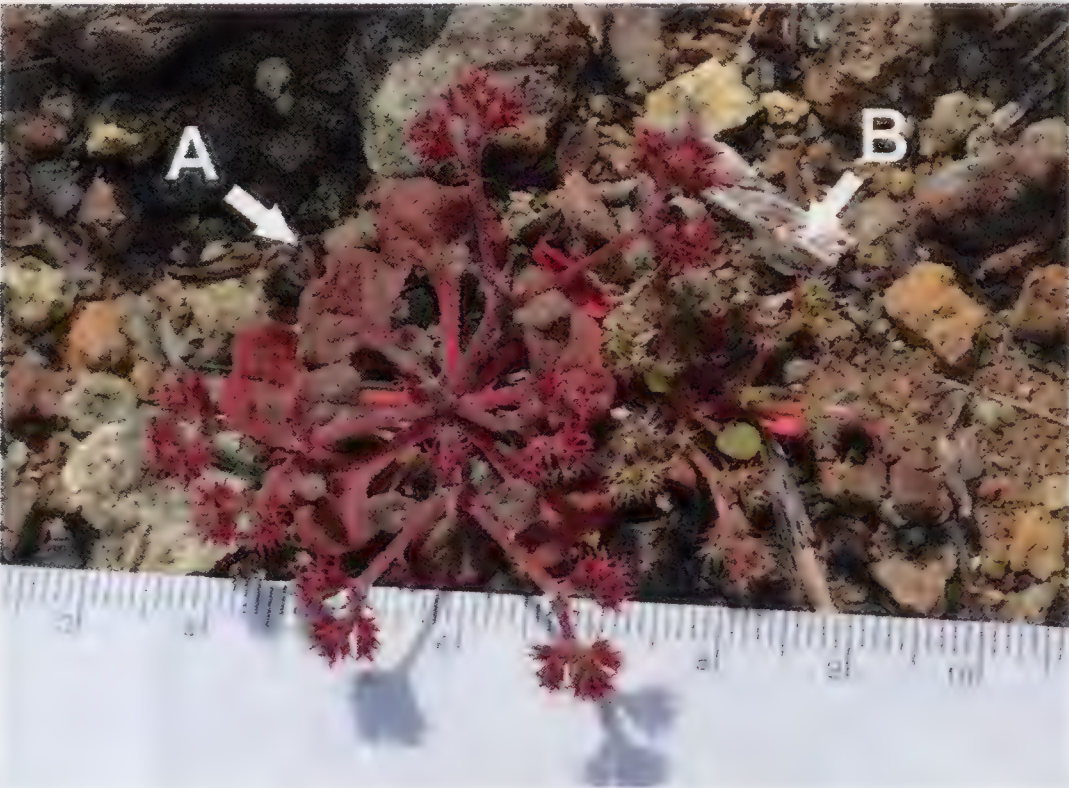


FIG. 2. *Chorizanthe aphanantha* and *C. breweri* growing together. (A) Individual of *C. breweri* in pre-flowering condition, with basal leaves present. (B) Individual of *C. aphanantha* in pre-flowering condition, with basal leaves present. Scale is in millimeters. Photo by K. M. Nelson.



FIG. 3. (A) Dwarfed, flowering individual of *C. aphanantha* adjacent to (B) similarly dwarfed individual of *C. breweri* with partially opened flowers. Scale is in millimeters. Photo by K. M. Nelson.



FIG. 4. Flowering branch of *C. aphanantha* with tight clusters of involucre. Photo by Kyle Nessen.

Annual, branched from base, 0.3–20 cm across; branches 0.2–10 cm, spreading to ascending; stems green or reddish-tinged when fresh, becoming red in age, puberulent with fine, curled, \pm appressed hairs 0.3–0.6 mm long. **Leaves** basal and at proximal nodes, more or less withered by anthesis; basal ovate to elliptic (occasionally \pm linear), basally tapered or occasionally truncate, apically obtuse to \pm rounded, occasionally mucronate; expanded blades (when present) 4–8 mm long, 0.6–5 mm wide, glabrous to minutely strigose, lateral veins obscure; petioles 2–10 mm long, sparsely strigose; proximal cauline leaves similar to basal, distal cauline generally linear, acuminate. **Inflorescences** dense, branched, not disarticulating at the nodes, bright green to reddish; bracts opposite, the proximal similar to vegetative leaves, but reduced, short-petiolate with narrow blade or linear and bladeless, distal 1.5–5 mm long, narrowly linear, straight, acrose with straight spine tips, ciliolate with stiff, ascending trichomes 0.2–0.4 mm long; involucre in tight, dichasial clusters, cylindric to narrowly obconic, not ventricose, 3-angled and 6-ribbed, tube 1.7–2.0 mm long, not or

faintly corrugate in age, ribs glabrous or distally sparsely strigillose, green, the narrow grooves between ribs with stiff, ascending or erect hairs 0.2–0.4 mm long, teeth alternately long and short, all tipped by uncinat awns, the longer spreading to ascending, 0.7–1 mm long (occ. 2 mm long on most proximal involucre), the shorter ascending to erect, about half as long, margins of teeth infolded, inconspicuously white-scarious. **Flowers** included in involucre or slightly exerted, pedicel \pm 0.2 mm; perianth cylindric, 1.5–1.7 mm long, throat greenish, glabrous, lobes monomorphic, 0.5–0.7 mm long, \pm ascending, oblong-ovate, entire, greenish-white, abaxially sparsely appressed-strigillose; stamens 9, the longest 3 with fully erect filaments, included or slightly exerted, shorter 6 with apically deflexed filaments; filaments distinct, adnate to base of hypanthium, \pm 1 mm long, ciliate at base; anthers rose-pink to purple, 0.2 mm long. **Achenes** lanceoloid, 1.7–2.0 mm long, shiny, smooth with 3 narrow ribs, light brown to dark reddish-brown, closely enwrapped by dried perianth.

Paratypes: CALIFORNIA, San Luis Obispo Co., San Luis Range, Irish Hills, Irish Hills Natural Reserve, Waddell Ranch acquisition, 35.252475°, –120.719412°, 312 m, ridgeline of a serpentine hill above From Creek with southern exposure, in open scrub habitat and at the margin of chaparral in rocky to gravelly soils, 2 Jun 2017, *K. M. Nelson s.n.* (OBI-83738); same locality, 13 Jun 2017, *K. M. Nelson s.n.* (OBI-83738); Irish Hills Natural Reserve, Waddell Ranch acquisition, 35.2449855°, –120.7023833°, 235 m, partially-shaded serpentine hill with eastern exposure, growing within and adjacent to trail at edge of chaparral shrub canopy, rocky to gravelly soils, 19 May 2018, *K. M. Nelson 44* (OBI); Irish Hills Natural Reserve, Waddell Ranch, 35.2429693°, –120.6973581°, 216 m, serpentine hill with eastern exposure, grassy meadow opening at the edge of chaparral shrub canopy, rocky to gravelly soils, 23 May 2018, *K. M. Nelson 47* (OBI); Irish Hills Natural



FIG. 5. Basal rosettes and early differentiation of inflorescences of (A) *C. breweri* and (B) *C. aphanantha*. Both images taken on April 05, 2018 by K. M. Nelson.

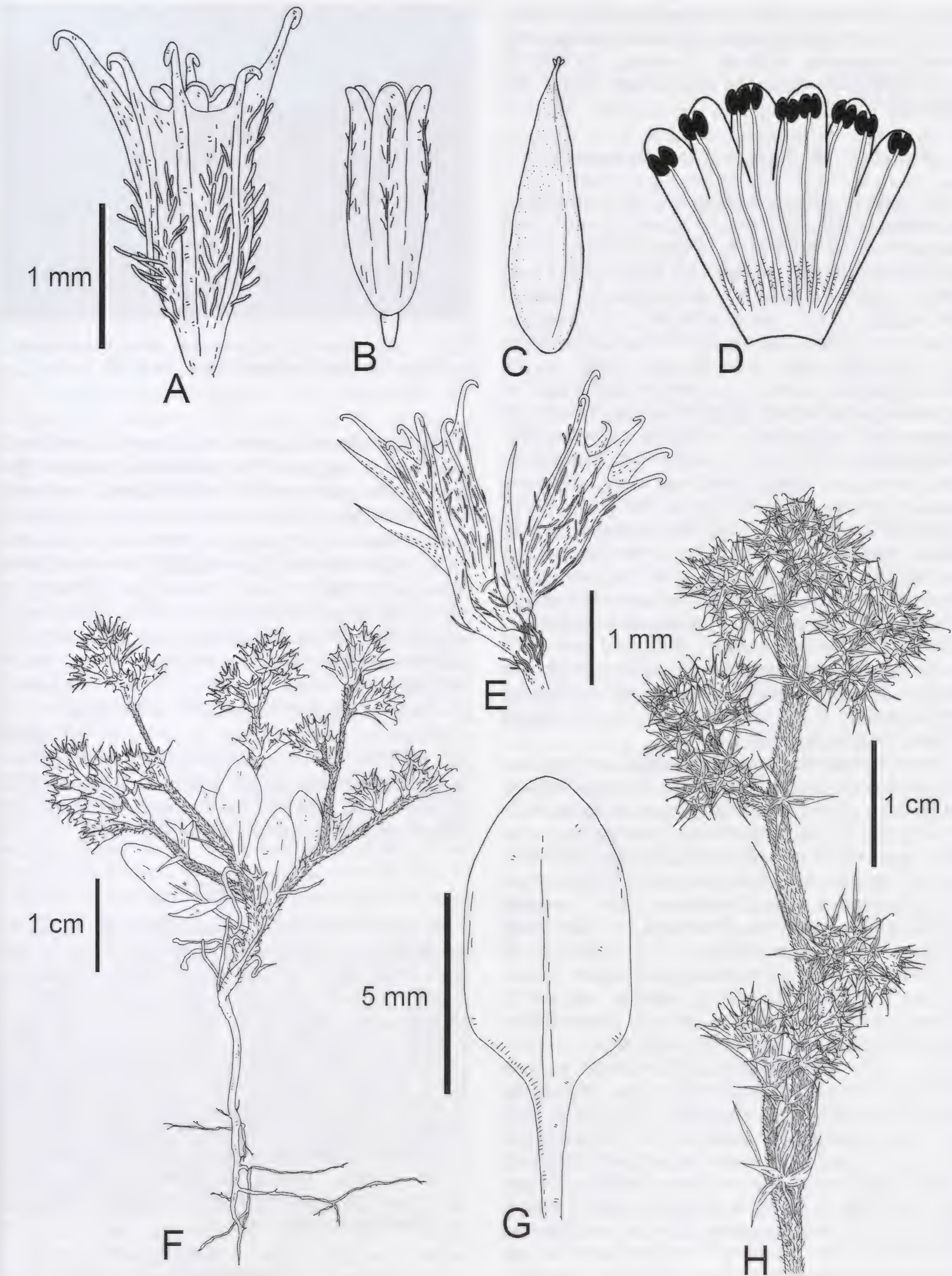


FIG. 6. *Chorizanthe aphanantha*: (A) individual involucre and tips of perianth lobes; (B) perianth with pedicel; abaxial perianth lobe faces with sparse, stiff, appressed trichomes along midveins; (C) achene tipped by withered stigmas and styles; (D) dissected perianth with attachment of filaments near base of perianth tube; proximal portion of filaments with spreading trichomes; (E) pair of involucres; inflorescence bracts stiffly ascending with straight tips; (F) individual plant showing dense, tufted habit and variation in shape of basal and proximal cauline leaves; (G) basal leaf; (H) mature inflorescence branch. A, E from photographs of dried specimens; B, C, D based on *Keil 35390*; F based on *Nelson 44*; G, H from photographs of live, rooted individuals. Illustrations by Nayl Gonzales.

Reserve, Mariposa Trail at junction with Durata Vista Trail, opening in area of serpentine chaparral, locally common with *C. breweri*, 35.25850°, -120.71499°, 230 m, 2 Jun 2018, David Keil 35390 (OBI, RSA, SD).

DISTRIBUTION, HABITAT, AND ABUNDANCE

Chorizanthe aphanantha is known to occur only in serpentine soils in areas of coastal scrub and chaparral in the Irish Hills Natural Reserve in the Irish Hills portion of the San Luis Range in San Luis Obispo County, California. Following the original discovery in 2017, *C. aphanantha* was only known to occur on a ridgeline and upper southeast-facing slope of a serpentine hill above Froom Creek, in the Waddell Ranch, a parcel that was recently added to the publicly-accessible Irish Hills Natural Reserve for conservation and passive recreation purposes. The Waddell Ranch is primarily surrounded by undeveloped open space areas comprising a mix of private ranch properties and public lands, including a parcel owned by the Bureau of Land Management (Fig. 1). Initial recognition that *C. aphanantha* was an undescribed species occurred near the end of its blooming period in 2017, and nearby areas of similar habitat had not yet been thoroughly surveyed. Additional fieldwork in April, May, and June 2018 in adjacent, similar habitats in the existing Irish Hills Natural Reserve revealed that several additional populations of *C. aphanantha* occur on city-owned property within the Irish Hills.

Local topography consists of steep, rocky slopes bisected by seasonal and perennial riparian corridors and grassy valleys. The underlying parent material is variably-weathered serpentinite. Vegetation varies from open scrub on south-facing slopes to locally dense chaparral on ridges and north-facing exposures. Occasional natural openings in the chaparral support grassy meadows, dominated by *Stipa lepida* Hitchc. and *Festuca perennis* (L.) Columbus & J.P.Sm., along with other annual, non-native grasses and native herbs. Dominant species on scrub-covered, south-facing slopes include *Hesperoyucca whipplei* (Torr.) Trel., *Artemisia californica* Less., and *Salvia mellifera* Greene, with scattered individuals of *Acmispon junceus* (Benth.) Brouillet var. *junceus* and *Diplacus aurantiacus* (Curtis) Jeps. Patches of bare soil occur among the shrubs and *Hesperoyucca* rosettes. The soils on slopes are shallow and very rocky. Chaparral stands are dominated by *Adenostoma fasciculatum* Hook. & Arn., *Ceanothus cuneatus* (Hook.) Nutt. var. *ramulosus* Greene, and *Quercus durata* Jeps. Other chaparral shrubs present include *Heteromeles arbutifolia* (Lindl.) M.Roem., *Pickeringia montana* Torr. & A.Gray, and, in some areas, *Arctostaphylos obispoensis* Eastw. Finer substrates occur near the ridgeline and in occasional flat areas, and may be locally covered by a layer of duff, consisting primarily of downed leaves of the nearby shrubs.



FIG. 7. Habitat of *C. aphanantha*, along margins of a rocky trail through chaparral. Photo by K.M. Nelson.

Chorizanthe aphanantha was found in locations scattered throughout the Irish Hills Natural Reserve within chaparral and scrub habitat, preferentially occupying trail margins (Fig. 7), edges of grassy meadow openings in the chaparral, and open, rocky terraces surrounding two abandoned chromium mines. It was documented growing in partial shade and in full sun on flat to moderately-sloped areas. It was frequently associated with the locally common *C. breweri* (Figs. 2, 3), which occupies the same habitats. It was observed growing as sparsely scattered individuals or in occasionally dense patches. In 2018, we estimated that the total known population comprised approximately 24,000 individuals. The current known area occupied by *C. aphanantha* is estimated to be about 2 hectares within a total 242-hectare area.

PHENOLOGY

In 2017, *Chorizanthe aphanantha* was found in late-flowering condition in early June, and in late flower and fruit in mid-June. During follow-up surveys in 2018, vegetative basal rosettes and early bolting were observed in early April, and open flowers were first noted in early May, with peak flowering occurring from mid to late May. Because of year-to-year variation in precipitation and temperature conditions, it might be expected to flower in some years by April or as late as the end of June. The co-occurring *C. breweri* may flower as early as mid-March, is commonly in full flower by late May, and may flower into early July.

ETYMOLOGY

The specific epithet *aphanantha* is derived from the Greek *aphanes* (unseen, invisible, obscure, secret) and *anthos* (flower), referring to plants characterized by very inconspicuous flowers.

Suggested common name: Irish Hills spineflower.

TAXONOMIC RELATIONSHIPS

Chorizanthe Benth. is a genus of about 50 species with a distribution in western North America and southwestern South America (Reveal and Hardham 1989; Reveal 2005; Reveal and Rosatti 2014). The genus is particularly well represented in California with 34 species currently recognized in the flora (Reveal and Rosatti 2014), 25 of which are California endemics. The most recently described species is the similarly small-flowered *Chorizanthe minutiflora* (Morgan et al. 2014), which is known from sandy coastal soils in northwestern Monterey County. In San Luis Obispo County, there are 19 known species, including *C. aphanantha* (D. J. Keil and R. F. Hoover, unpublished data). In addition to *C. aphanantha*, three species of *Chorizanthe* – *C. breweri*, *C. palmeri* S.Watson, and *C. ventricosa* Goodman – are wholly or largely restricted to soils derived from serpentine or serpentinized igneous deposits (Reveal 2005); all three grow in San Luis Obispo County, and *C. breweri* is endemic to the County. *Chorizanthe breweri* and *C. palmeri* both occur in the Irish Hills, and *C. breweri* often grows in immediate proximity to *C. aphanantha*, sometimes with overlapping canopies (Figs. 2, 3). Though co-occurring in the same habitat, *C. breweri* and *C. palmeri* are easily distinguished from *C. aphanantha*, as described below, and they do not appear to be the closest relatives of *C. aphanantha*.

Most California species of *Chorizanthe* are members of *Chorizanthe* subg. *Amphietes* Reveal and Hardham sect. *Ptelosepala* Nutt. (Reveal 2005), and *C. aphanantha* clearly is a member of this section. Based on the narrow white margins of the involucre bracts, *C. aphanantha* would key to subsect. *Pungentes* Goodman (Reveal and Hardham 1989). In flower size, *C. aphanantha* most closely resembles the recently described *C. minutiflora*. *Chorizanthe aphanantha* may be distinguished from *C. minutiflora* by its much sparser and shorter pubescence, entire rather than erose and cuspidate perianth lobes, and nine rather than three stamens. *Chorizanthe aphanantha* also resembles small-flowered individuals of *C. procumbens* in having green involucre and entire perianth lobes, but differs in its ovate to elliptic rather than oblong to oblanceolate leaf blades, its sparser and shorter pubescence, the presence of a narrow scarious margin on the involucre teeth, \pm glabrous involucre ribs, and filaments that are distinct rather than basally connate. Although there is morphological variability among geographically disjunct populations of *C. procumbens* (Russell 2003; Reveal and Hardham 1989), none of these morphological variations approach *C. aphanantha*. *Chorizanthe procumbens*, a member of subsect. *Procumbentes* Goodman (Reveal and Hardham 1989), is probably only distantly related to *C. aphanantha*.

In vegetative form, the basal leaf morphology of *C. aphanantha* is easily distinguished from that of the co-occurring *C. breweri*. In particular, *C. aphanantha* leaf blades are ovate to elliptic (occasionally mucronate) and glabrous to minutely strigose-hairy with obscure veins, whereas *C. breweri* basal leaves are reniform to orbiculate (\pm obcordate), generally apically notched, and puberulent to tomentose with prominent veins (Figs. 2, 5, 6F, 6G). In flowering condition, *C. aphanantha* is easily distinguished from *C. breweri* by its generally much smaller stature and compact growth form (dwarfed individuals of both may be similar in size [Fig. 3]), by the green rather than reddish cast of its involucre, and by its minute flowers (Figs. 2, 3). However, the entire plant body of late-season individuals of both species turns reddish brown, and plants of *C. aphanantha* then become more difficult to distinguish from small individuals of *C. breweri*. Late-season plants of *C. aphanantha* may be distinguished from *C. breweri* by erect (Fig. 6E) vs. recurved inflorescence bracts, shorter involucre tubes (1.5–1.7 mm vs. 2–3 mm), and by smooth vs. transversely corrugated mature involucre ribs. As noted above, *C. aphanantha* is a member of subsect. *Pungentes*, whereas *C. breweri* is a member of subsect. *Staticoideae* Goodman.

Of the remaining serpentine-endemic species, *Chorizanthe palmeri* is easily distinguished from *C. aphanantha* by its erect growth form, and red to purple, dimorphic perianth lobes. Similarly, *C. ventricosa* may easily be distinguished by its red to maroon, dimorphic perianth lobes, and by having a straight abaxial involucre awn, whereas *C. aphanantha* has all hooked awns. Both *C. palmeri* and *C. ventricosa* are members of Subsection *Petasata*.

In addition to our field surveys in 2017 and 2018, we searched collections of *Chorizanthe* in the Robert F. Hoover Herbarium on the campus of California Polytechnic State University, San Luis Obispo, for mis-identified specimens of *C. aphanantha*. Specimens inspected included collections of *C. breweri*, *C. obovata* Goodman, *C. palmeri*, *C. diffusa* Benth., *C. spinosa* S.Watson, *C. uniaristata* Torr. & A.Gray, *C. ventricosa*, *C. watsonii* Torr. & A.Gray, and *C. staticoides* Benth., as well as specimens lacking a positive identification. No collections of *C. minutiflora* or *C. procumbens* are deposited at the Hoover Herbarium. No mis-identified specimens were discovered. With the discovery of *C. aphanantha* in mind, we also visited several other serpentine areas in the San Luis Obispo area, but did not find any other occurrences.

Flowering individuals of *Chorizanthe* species that grow in the San Luis Range may be distinguished with the following key. Because the narrow scarious margins of its involucre bracts may be easily overlooked, particularly in dry specimens, we have included *C. aphanantha* under both leads in the first key couplet.

KEY TO SPECIES OF *CHORIZANTHE* IN THE SAN LUIS RANGE

- 1. Involucre teeth with scarious or membranous margins
 - 2. Membranous margin continuous across sinuses connecting involucre teeth *C. membranacea*
 - 2' Membranous margin narrowed toward bases of individual involucre teeth or \pm equally narrow entire length
 - 3. Membranous margins of involucre teeth conspicuous *C. diffusa*
 - 3' Membranous margins of involucre teeth inconspicuous
 - 4. Perianth tube and throat white; lobes white to rose, tips jagged; perianth 2.5–3 mm . . . *C. angustifolia*²
 - 4' Perianth tube, throat, and lobes greenish-white; lobes entire; perianth 1.5–1.7 mm . . . *C. aphanantha*²
- 1' Involucre teeth without scarious or membranous margins
 - 5. Inner perianth lobes fringed or bilobed
 - 6. Perianth generally white or slightly pink-tinged; stems, except in the smallest individuals generally \pm prostrate *C. obovata*
 - 6' Perianth red to purple; stems generally erect *C. palmeri*
 - 5' Inner perianth lobes entire
 - 7. Perianth 1.5–1.7 mm long; involucre green at flowering *C. aphanantha*²
 - 7' Perianth 2–5 mm long; involucre red to purple at flowering
 - 8. Stems erect or ascending, generally leafless *C. staticoides*
 - 8' Stems decumbent or prostrate, generally proximally leafy
 - 9. Mature involucre not transversely corrugate *C. angustifolia*²
 - 9' Mature involucre transversely corrugate *C. breweri*

SERPENTINE ENDEMISM

Serpentine soils present significant challenges to plant growth, due largely to the unusually high concentration of magnesium and low concentration of calcium (low calcium to magnesium ratio), the presence of toxic trace elements, and the generally low nutrient content and water-holding capacity of these soils (Safford et al. 2005). As a result of the harsh growing conditions, serpentine soils are known to support stunted vegetation and a high degree of endemism (Safford et al. 2005; Harrison and Rajakaruna 2011; Anacker 2014). Though generally uncommon, serpentine soils occur frequently in San Luis Obispo County, and they are the predominant soil type in the Irish Hills Natural Reserve. In general, the patchy distribution of serpentine soils presents significant opportunities for the evolution of rare, often locally endemic species. In San Luis Obispo County, and in the Irish Hills in particular, the extent of relatively large serpentine islands is sufficient to support and maintain populations of locally endemic, edaphic specialists, such as *C. aphanantha*. Several other rare and endangered serpentine-endemic taxa are known to occur within the Irish Hills Natural Reserve and nearby areas of the San Luis Range (California Native Plant Society 2018): *Arctostaphylos obispoensis* Eastw., *Calochortus obispoensis* Lemmon, *Calochortus clavatus* S.Watson var. *clavatus*, *Carex obispoensis* Stacey, *Chorizanthe breweri*, *Chorizanthe palmeri*, *Cirsium fontinale* (Greene) Jeps. var. *obispoense* J.T.Howell, *Dudleya abramsii* Rose subsp. *murina* (Eastw.) Moran, and *Monardella palmeri* A.Gray. Most of these species were observed on the Waddell Ranch property and in other sites in the Irish Hills, occupying habitats similar to those of *Chorizanthe aphanantha*.

Though *C. aphanantha* is currently only known to occur in the Irish Hills portion of the San Luis Range, it is possible that additional localities are present in nearby serpentine islands in San Luis Obispo or perhaps adjacent counties, as is the case for the other serpentine-endemic species listed above.

PRIOR LAND USE, CONSERVATION STATUS, AND THREATS

The Waddell Ranch property has been placed in permanent conservation status under the new management of the City of San Luis Obispo, along with the rest of the Irish Hills Natural Reserve. Past known land uses on the Waddell Ranch property and adjacent areas include livestock grazing, chromite mining, and public recreation. A network of recreational trails exists throughout city-owned portions of the Irish Hills, where *C. aphanantha* was found. *Chorizanthe aphanantha* was observed preferentially occupying transitional habitats, primarily at the edge of chaparral shrub canopy and often along trail margins. Although populations occurring under a dense chaparral canopy would be more difficult to detect, the 2018 surveys included areas of both open, transitional, and densely-vegetated chaparral habitat throughout the Irish Hills Natural Reserve, and no populations were found under dense shrub cover. Based on these observations, it appears likely that *C. aphanantha* is tolerant of low to moderate levels of disturbance. There is no recorded fire history for the occupied habitat area or within the larger Irish Hills region (California Department of Forestry and Fire Protection 2017). Therefore, the discovery of *C. aphanantha* is not linked to any recent fire history, and the response of this species to fire is unknown.

We request that the California Native Plant Society review *Chorizanthe aphanantha* for rarity

and inclusion in the Inventory of Rare and Endangered Plants of California (California Native Plant Society 2018). Although the area of the Waddell Ranch/Irish Hills Natural Reserve where *C. aphanantha* occurs is under permanent conservation, uncertainty of future impacts to the population such as prolonged drought, catastrophic wildfire, or unintended anthropogenic impacts remain, and the fact that the species has just one known occurrence, justify consideration for inclusion in the Inventory of Rare and Endangered Plants of California.

ACKNOWLEDGMENTS

We thank the City of San Luis Obispo for their visionary efforts to protect a greenbelt of permanent conservation lands surrounding the city and, in particular, the City's Open Space Team for their inspiring work to bring together local landowners, conservationists, academics, and government officials to create a plan for the future of San Luis Obispo's Greenbelt. We also thank the Waddell family for their past stewardship of the property and their decades of planning effort to ensure it was placed into conservation. Thanks to the dedicated staff and volunteers of the Robert F. Hoover Herbarium, who have maintained such a valuable resource for regional botanical discoveries and research, and further made this resource accessible during the preparation of this manuscript. Drs. Matt Ritter and Jenn Yost, Brooke Langle, and Kyle Nessen assisted in field-verifying the population. Illustrations were prepared by Nayl Gonzales. Thank you to Drs. Matt Ritter, Jenn Yost, and Nishi Rajakaruna for your helpful comments on early drafts the manuscript.

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TWO NEW SEDGES (*CAREX* SECT. *ACROCYSTIS*, CYPERACEAE) FROM
NORTHWESTERN MEXICO AND SOUTHERN CALIFORNIA

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ABSTRACT

Carex bajacalifornica Zika is described from five populations in the mountains of northern Baja California, Mexico, in the Sierra de Juárez and Sierra de San Pedro Mártir. It is distinguished from *Carex rossii* Boott by its short inflorescence bract, abbreviated perigynium beak shorter than the stipe, and nerved perigynium faces. *Carex cryptosperma* Zika, D.S. Bell, and L.J. Gross is described from 12 populations in southern California, in the San Bernardino, San Gabriel, and San Jacinto Mountains. *Carex cryptosperma* differs from *Carex xerophila* Janeway & Zika in its relatively short perigynium stipe and relatively long perigynium beak. The new species are illustrated and mapped, and a key is presented to members of *Carex* sect. *Acrocystis* in the states of California and Baja California.

RESUMEN

Carex bajacalifornica Zika se describe a partir de cinco poblaciones en las montañas del norte de Baja California, México, en la Sierra de Juárez y la Sierra de San Pedro Mártir. Se distingue de *Carex rossii* Boott por su bráctea de inflorescencia corta, pico de perigynium abreviado más corto que el estípide y caras de perigynium nervadas. *Carex cryptosperma* Zika, D.S. Bell y L.J. Gross se describe a partir de doce poblaciones en el sur de California, en las montañas de San Bernardino, San Gabriel y San Jacinto. *Carex cryptosperma* difiere de *Carex xerophila* Janeway & Zika en su estípide de perigynium relativamente corto y su pico de perigynium relativamente largo. Las nuevas especies se ilustran y mapean, y se presenta una clave a los miembros de la *Carex* secta. *Acrocystis* para los estados de California y Baja California.

Key Words: *Carex bajacalifornica*, *Carex cryptosperma*, *Carex* section *Acrocystis*, Cyperaceae, endemic, taxonomy.

Botanists were long vexed by reports of disjunct populations of *Carex pityophila* Mack. and *C. geophila* Mack. in California (Crins and Rettig 2002), as well as reports of *C. globosa* Boott from northwest Baja California in Mexico (Hermann 1974; Rebman and Roberts 2016). These sedges are three members of *Carex* section *Acrocystis*, a complex group of morphologically similar species in North America, whose specimens are often misidentified and thus their distributions are still imperfectly known. Most of those reports were referred to as *Carex rossii* Boott and *C. deflexa* Hornem. var. *boottii* L.H.Bailey in the recent California flora (Zika et al. 2012, 2014). However, some collections were not easily placed. Those gatherings were from the mountains of northwest Mexico and adjacent California, with heavily nerved perigynia that suggested, but did not match, *Carex globosa*. Nor did they align with recently described members of the section from further north in California, *C. serpenticola* Zika (Zika et al. 1998) and *C. xerophila* Janeway & Zika (Zika et al. 2014). We describe them here as previously unrecognized species.

TAXONOMIC TREATMENT

***Carex bajacalifornica* Zika, sp. nov.** (Fig. 1) TYPE: MEXICO, Baja California, Sierra San Pedro Mártir, *Pinus jeffreyi* Grev. & Balf. forest above Observatory living quarters, elev. circa 2600 m, 7 May 1986, Robert F. Thorne 61972 & T. S. Elias, P. Rojas (holotype: RSA353942; isotypes: BCMEX2608 n.v., CAS816834, UCR44045).

Species haec a Carice globosa spicis basalibus erectis brevibus necnon culmis brevioribus recedit, a Carice rossii bractea inflorescentiae infima brevi, perigyniis obovatis nervosis et rostro stipite brevior statim dignoscenda.

Plants densely to loosely cespitose, rhizomes internodes 0–4 cm, forming tufts. Proximal sheaths reduced to scales, bladeless, dark purple, smooth to scabrous on the prominent nerves, the veins generally persisting as fibers at the plant base, or as ladder-fibrillose sheath fronts. Distal sheaths with translucent fronts, red- or green-veined, apices V-shaped; ligules obtuse, mostly longer than wide, margins scabrous; distal sheaths bearing blades. **Leaves** in

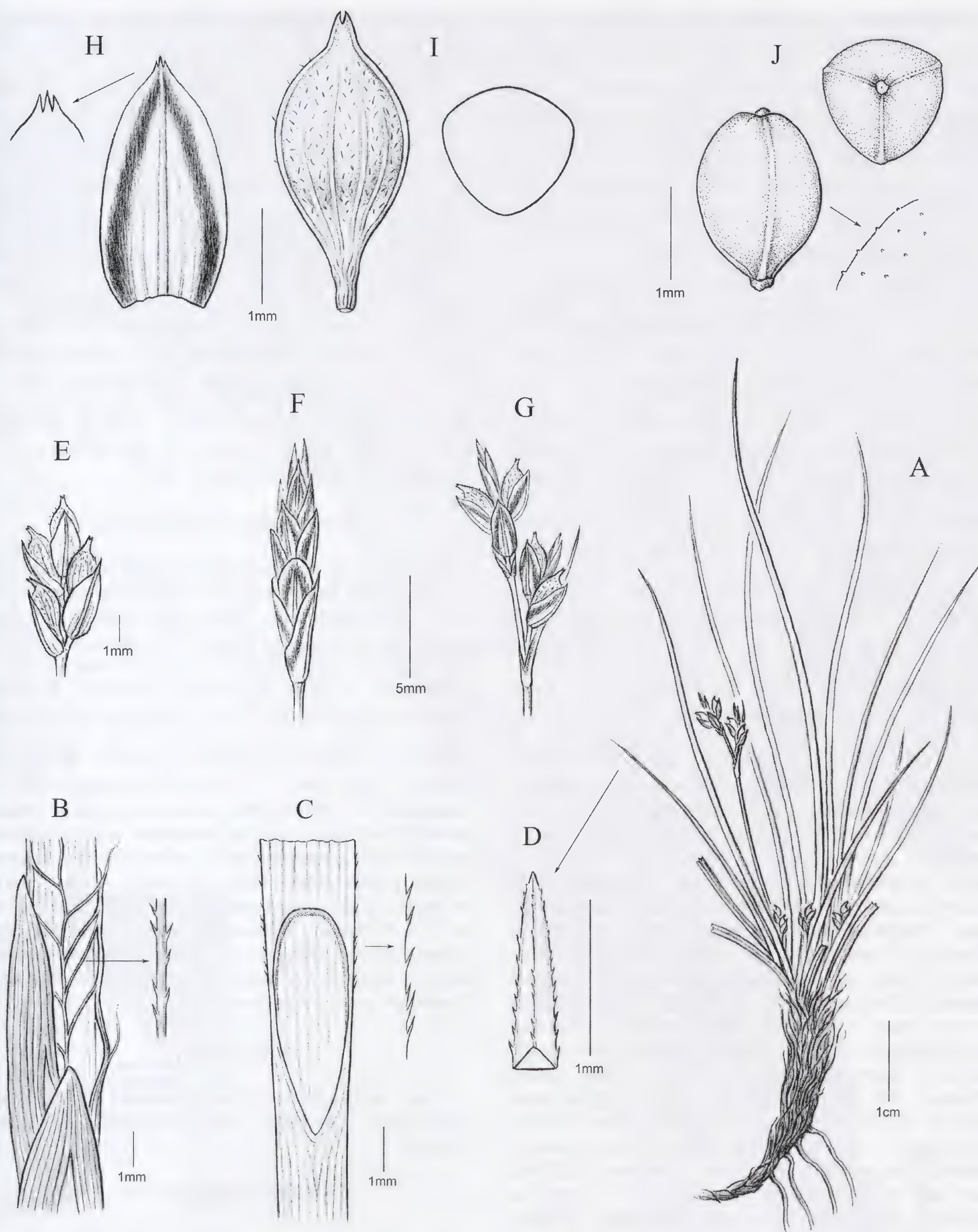


FIG. 1. *Carex bajacalifornica*. A. Habit. B. Sheath front showing persisting scabrous fibers. C. Sheath apex and ligule, showing scabrous proximal blade margin. D. Triangular scabrous leaf tip. E. Basal spikelet. F. Staminate spike. G. Non-basal inflorescence on elongate stem, showing inconspicuous inflorescence bract. H. Pistillate scale, with detail of notched and mucronate apex. I. Pubescent perigynium, dried, showing beak shorter than stipe, and bluntly 3-sided equatorial cross-section. J. Achene, side view with surface detail, and top view. A, C–D, G–J. Type, Sierra de San Pedro Mártir, Baja California, *Thorne 61972 & Elias, Rojas*. B. Sierra de Juárez, Baja California, *Thorne 55852 & Wisura, Steinmetz, et al.* E. Sierra de San Pedro Mártir, *Kentner 233 & Singh, Hickenbottom, Williams*. F. Sierra de San Pedro Mártir, *Kentner 266 & Williams, Wiggins, Richter*.

sterile rosettes; stem leaves 1–3 on proximal 1/4 of fertile stems, most blades taller than fertile stems, blades 2–20.5 cm long, 1.3–2.4 mm wide, flat to shallowly V- or W-shaped in cross-section, sometimes folded, veins 5–9, prominent, adaxial surface smooth to slightly scabrous distally, blade margins scabrous, apices filiform-triangular, densely scabrous, discolored distally, acute to acuminate, bristly (visible at 20× magnification). **Stems** (culms) 1–6.5 cm tall, triangular in cross section, usually scabrous distally. **Basal inflorescences:** spikes 1–3, 4–7 mm long, sessile or on erect slightly scabrous peduncles to 5 mm, 0.2–0.3 mm wide, with 2–5 perigynia, pistillate (Fig. 1E). **Non-basal portion of inflorescence:** 9–15 mm long, on elongate stems, with 1–3 spikes, the proximal pistillate (rarely androgynous), 4–6 mm long, with 2–4 perigynia (Fig. 1G), sessile or on peduncles to 4 mm long; overtopped by foliage; lowest non-basal inflorescence bract translucent, green, or red-brown at base, midvein green, blade flat to slightly folded, at least distally, weakly 1–3 veined, scabrous distally, scarcely leaf-like, often mostly scarious, 7–11 mm long, shorter than inflorescence (with rare exceptions on atypical late season shoots), apex filiform-triangular; distal spike staminate, 5–13 mm long, 0.9–2.4 mm wide, 3–11 flowered (Fig. 1F), often inconspicuous; stamens 3 per scale, filaments white, anthers yellow, linear-oblong, 2.3–2.5 mm long, apical appendage 0.1 mm long, not bristly. **Staminate scales:** 2.5–5.3 mm long, midvein green, bordered with inconspicuous to prominent red-brown bands at maturity, the translucent margins 0.1–0.4 mm wide, smooth or scabrous on the prominent or keeled midvein, with 6–8 inconspicuous fine parallel lateral veins, apex of proximal scales tending to be blunt to truncate, of distal scales tending to be acute to acuminate, or slightly notched and mucronate, the mucro to 0.5 mm. **Pistillate scales:** oblong-obovate to oblong, clasping at base, 2.6–4.5 mm long, 1.3–2.0 mm wide, shorter than or occasionally subequal to the perigynia, glabrous, smooth or slightly scabrous, green central band with 1(–3) strong midveins, the midveins slightly or strongly raised, forming a smooth to slightly scabrous distal keel, the green portion flanked with wide red-brown to dark purple bands and up to 6 inconspicuous fine parallel veins, scarious margins 0.1–0.3 mm wide, distal margins usually entire, apex acute to acuminate, sometimes shallowly notched and mucronate, the mucro to 0.6 mm and smooth to scabrous or bristly. **Perigynia:** obovate, pubescent, 2.9–3.7 mm long, 1.2–1.6 mm wide; fresh stipes presumed to be fleshy, oily, turgid and pale; dried stipes shriveled, 0.6–1.1 mm long, longer than the beak; body plump, rounded-trigonous, usually with $3-5 \pm$ prominent veins on 1 or more faces when mature; beak distinct, only slightly flattened, erect or slightly incurved, slightly pubescent, the margins subentire, not scabrous, 0.35–0.8 mm long (measured from inflection point), including white teeth 0.1–0.2 mm long, 3-styled. **Achenes** broadly ovoid to oblong-

ovoid, rounded-trigonous, surface honeycomb-reticulate, papillose (40×), brown, 1.8–2.0 mm long, 1.3–1.4 mm wide.

Paratypes: MEXICO, **Baja California:** Parque Nacional Sierra de San Pedro Mártir, circa 2.25 km NE of park entrance on main paved road to the observatory, 2387 m, 6 May 2013, *E. Kentner 233 & O. Singh, M. Hickenbottom, J. Williams* (RSA) [mixed collection with immature *Carex praeegracilis* W.Boott]; Parque Nacional Sierra de San Pedro Mártir, vicinity of Botella Azul, near the trail to Picacho del Diablo, 2670 m, 3 Jun 2013, *E. Kentner 266 & J. Williams, H. Wiggins, C. Richter* (RSA); Vallecitos, near road to observatory and campground, 2430 m, 18 Jun 1985, *R. F. Thorne 60856 & R. Dahlgren, S. Boyd, D. Charlton* (RSA); Constitucion National Park, Sierra de Juárez, ridge W of N end of Laguna Hanson, 1650 m, 28 May 1983, *R. F. Thorne 55852 & W. Wisura, W. Steinmetz, et al.* (CAS, RSA).

DISTRIBUTION AND ECOLOGY

We have not seen populations of *Carex bajacalifornica* in the field, and so we can only summarize data from herbarium labels. The earliest known gathering was made by Robert Thorne et al. in 1983. The localities (Fig. 2) span 130 km of the northern mountains in Baja California, Mexico. Known elevations are 1650–2670 m, but we have no microsite data for slope or soil moisture. The underlying bedrock in some locations is granitic. Recorded habitats are open or mixed coniferous forest, dominated by Jeffrey pine, sometimes with a sparse shrubby understory. Other associated vascular plants include *Abies concolor* Lindl., *Arctostaphylos pungens* Kunth, *Carex praeegracilis*, *Eriogonum wrightii* Torr. ex Benth., *Leptosiphon melingii* (Wiggins) J.M.Porter & L.A.Johnson, *Monardella macrantha* A.Gray, *Pinus contorta* Douglas ex Loudon, *Populus tremuloides* Michx., *Quercus chrysolepis* Liebm., and *Symphoricarpos longiflorus* A.Gray.

PHENOLOGY

Carex bajacalifornica fruits matured in May and early June. The plants were unidentifiable before fruiting.

ETYMOLOGY

Carex bajacalifornica, or Baja sedge, is an endemic named for Baja California, Mexico.

SIMILAR SPECIES

Carex bajacalifornica is assigned to *Carex* sect. *Acrocystis* Dumort. (Crins and Rettig 2002) based on its dense growth form and pubescent perigynia in compact pistillate spikes. Among the species with basal spikes, it was initially confused with *C. rossii*



FIG. 2. Distribution of *Carex bajacalifornica* and *C. cryptosperma*, based on verified herbarium specimens.

and *C. globosa* in the herbarium. We did not find mixed collections in the specimens we examined. *Carex bajacalifornica* is separable from the common and widespread *C. rossii* by its nerved obovate perigynia with a beak shorter than the stipe, and the inconspicuous inflorescence bract which is shorter than the inflorescence. *Carex rossii* perigynia are more elliptical, have strong marginal ribs (but no additional nerves on the perigynium faces), the beaks equal to or longer than the stipe, and the inflorescence bract is conspicuous, leaf-like, and exceeds the inflorescence.

Carex bajacalifornica differs from *C. globosa* because its basal spikes are supported by stiffly erect and very short or sessile peduncles. *Carex globosa* basal spikes have much longer and ultimately arching peduncles. The two also are separable by stem length and perigynia width. *Carex bajacalifornica* has fertile stems 1–6.5 cm, and perigynia $2.9\text{--}3.7 \times 1.2\text{--}1.6$ mm. *Carex globosa* has fertile stems 7–47 cm, and perigynia $3.5\text{--}5 \times 1.6\text{--}2.3$ mm.

The generally smaller parts and more southern range separate *Carex bajacalifornica* from *C. xerophila*, a species that shares with it a heavily nerved perigynium and beak shorter than the stipe. *Carex bajacalifornica* fertile stems are 1–6.5 cm tall, and the slender basal spikes are sessile or on peduncles 1–5 mm. *Carex xerophila* stems are 9.5–35 cm tall, and the broad basal spikes are on peduncles 10–75 mm. The two are allopatric, separated by 850 km (Zika et al. 2014).

Differences with other members of the section in our region are summarized in the key below. In section *Acrocystis*, there are several additional species with pistillate spikes at basal nodes, which are known from further north and east in North America, such as *C. geophila*, *C. tonsa* (Fernald) E.P. Bicknell, and *C. umbellata* Schkuhr ex Willd. All of these extralimital taxa with basal spikes differ from *C. bajacalifornica* in their unnerved perigynium faces, among other characters [see the keys of Crins and Rettig (2002)]. *Carex geophila* can have some nerves present at the base of the perigynia (Crins and Rettig 2002), but the fresh foliage is blue-green, the plant base is more fibrous, and the perigynium beak is longer than in *C. bajacalifornica*.

SPECIES INCERTAE SEDIS

Certain specimens collected from the Sierra de San Pedro Mártir and Sierra Blanca, may or may not be *Carex bajacalifornica*. These plants were stunted, presumably by drought, and had immature or aborted perigynia. They could be *C. rossii*, a common montane species in southern California, and reported from northern Baja California (Rebman and Roberts 2016). We verified three *C. rossii* vouchers (with unnerved perigynia and long leaf-like inflorescence bracts) from the observatory area in the Sierra de San Pedro Mártir (Rebman 4166 et al. BCMEX, SD; Thorne 60895 et al. RSA; Thorne 63757 et al. RSA,

SD). To separate *C. bajacalifornica* and *C. rossii* morphologically, mature fruits and infructescences are required. Four undetermined specimens, originally labeled as *C. globosa* or *C. brevipes* W. Boott (a synonym of *C. rossii*), are listed below, and we encourage collectors with permits to investigate these populations and gather specimens with ripe perigynia, so their identity can be resolved. Moran 17592 was reported by Hermann (1974) as *C. globosa*, based on a SD specimen we have not seen.

MEXICO, Baja California: [Sierra de San Pedro Mártir], El Picacho del Diablo, 3095 m, 5 May 1978, R. Moran 25601 (SD); [Sierra de San Pedro Mártir], Yerba Buena, common in *Pinus jeffreyi* forest, elev. circa 2475 m, 16 Aug 1967, R. Moran 14134 & R. F. Thorne (RSA); [Sierra de San Pedro Mártir], occasional on steep east slope of Cerro “2828” on east rim, elev. circa 2800 m, near $31^{\circ} 02' \text{ N}$, $115^{\circ} 27' \text{ W}$, 6 Jul 1968, R. Moran 15277 (RSA); [Sierra Blanca], common in Coulter pine wood, on upper N slope of Cerro Blanco, elev. circa 1200 m, near $32^{\circ} 03' \text{ N}$, $116^{\circ} 30' \text{ W}$, 24 May 1970, R. Moran 17592 (RSA).

Carex cryptosperma Zika, D.S. Bell, and L.J. Gross, sp. nov. (Fig. 3) TYPE: USA, California, San Bernardino Co., San Gorgonio Wilderness, South Fork Trail, understory of burnt forest, 2379 m, 31 May 2016, Duncan S. Bell 9660 and LeRoy J. Gross (holotype: RSA; isotypes: CAS, GH, MO, NY, WTU).

Species haec a Carice rossii perigyniis cum nervis amphifacialibus, necnon nervis lateralibus in glumis femineis differt. A Carice xerophila perigyniis cum rostro stipite brevior recedens.

Plants densely to loosely cespitose, rhizome internodes to 5.5 cm, forming tufts. Proximal sheaths reduced to scales, bladeless, reddish-brown, smooth to scabrous on the prominent nerves, a few veins sometimes persisting as fibers at the plant base, or as ladder-fibrillose sheath fronts. Distal sheaths with translucent fronts, often red-veined, apices concave to subtruncate; ligules obtuse, mostly longer than wide, margins scabrous; distal sheaths bearing blades. **Leaves** in numerous sterile rosettes; stem leaves 6–9 on proximal 1/3 of fertile stems, some blades taller than fertile stems, blades 4–16 mm long, 1–1.9 mm wide, shallowly V- or W-shaped in cross-section, sometimes folded, green not blue-green, with 5–10 prominent veins, adaxial surface scabrous, blade margins scabrous, apices filiform-triangular, densely scabrous, distally blunt or truncate, bristly (20×). **Stems** 3.5–16 cm tall, triangular, scabrous. **Basal inflorescences:** spikes 1–4, 7–12 mm long (Fig. 3E), on erect scabrous peduncles 0.3–2.3 cm long, 0.2–0.55 mm wide, with 2–6 perigynia, mostly pistillate, occasionally androgynous with 2–3 terminal staminate scales. **Non-basal portion of inflorescence:** on elongate stems, with 1–3 spikes, the proximal pistillate, 5.5–8 mm long, with 2–4 perigynia (Fig. 3F), sessile or on peduncles to 3 mm long; overtopped by foliage; lowest non-basal inflorescence

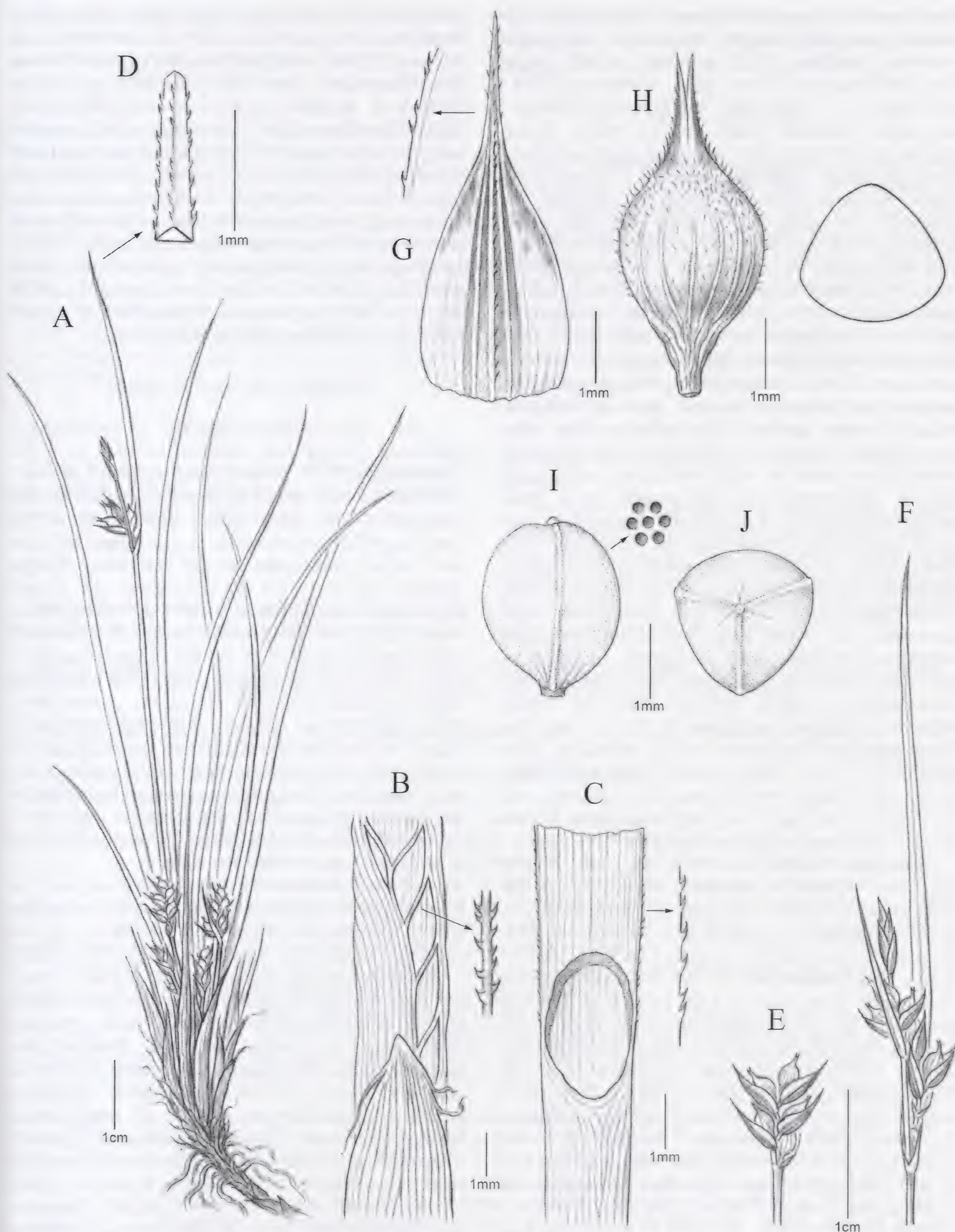


FIG. 3. *Carex cryptosperma*. A. Habit. B. Sheath face showing persisting scabrous fibers. C. Sheath apex and ligule, showing scabrous proximal blade margin. D. Triangular scabrous leaf tip. E. Basal spikelet. F. Non-basal inflorescence on elongate stem, showing elongate leaf-like inflorescence bract. G. Pistillate scale, showing scabrous awn. H. Pubescent perigynium, dried, showing beak longer than stipe, and bluntly 3-sided equatorial cross-section. I. Achene, side view and surface detail. J. Achene, top view. A, D–J. Type, San Bernardino Co., California, *Bell 9660 & Gross*. B–C. San Bernardino Co., California, *Bell 9670 & Gross*.

bract green at base, blade green, slightly keeled at least at base, otherwise flat, with several strong ribs, scabrous, leaf-like, 17–70 mm long, mostly longer than inflorescence; distal spike staminate, 6.5–8.5 mm long, 0.9–1.5 mm wide, 4–9 flowered; stamens 3 per scale, filaments white, anthers yellow, linear-oblong, 2.2–2.3 mm long, apical appendage 0.1 mm long, not bristly. **Staminate scales:** 3.1–5.1 mm long, green, bordered with red-brown at maturity, the translucent margins 0.2–0.5 mm wide, sometimes scabrous, with prominent or keeled pale midveins, and 4–8 usually inconspicuous fine parallel lateral veins, apex blunt to acuminate, occasionally mucronate, mucro to 0.3 mm. **Pistillate scales** ovate-oblong to oblong, clasping at base, 3.9–6 mm long, 1.3–1.8 mm wide, usually shorter than perigynia but on basal spikes occasionally longer than perigynia, glabrous, scabrous, or sparsely pubescent, green central band with 1–3 strong midveins, the midveins often raised or forming a keel and scabrous, the non-green portion translucent or with a red-brown lateral band, with 6–10 inconspicuous fine parallel veins, distal margins entire to erose or sparsely short-ciliate, apex acute to truncate, if mucronate, the mucro to 0.1 mm and scabrous or ciliate. **Perigynia** elliptic to obovate, pubescent, 3.7–4.7 mm long, 1.5–2.1 mm wide; fresh stipes fleshy, oily, turgid and pale, dried stipes shriveled, 0.4–1.5 mm long; body plump, rounded-trigonous, at least some perigynia with 3–5 \pm prominent veins on 1 or more faces when mature; beak distinct, slightly flattened, erect or slightly incurved, scabrous and pubescent, 0.6–1.7 mm long (measured from inflection point), including white teeth 0.2–0.6 mm long; 3-styled. **Achenes** oblong-ovoid to oblong-obovoid, rounded-trigonous, surface reticulate (Fig. 3I) or papillose (40 \times), brown, 2.1–2.7 mm long, 1.6–1.7 mm wide.

Paratypes: USA, CALIFORNIA, **Los Angeles Co.:** San Gabriel Mountains, Blue Ridge, 2438–2591 m, 18 Jul 1947, *J. T. Howell* 23393 (CAS n.v., RSA). **Riverside Co.:** San Jacinto Mountains, Lake Fulmor, 17 May 1959, *P. H. Raven* 14228 (CAS, GH, RSA). **San Bernardino Co.:** San Bernardino Mountains, Bluff Lake, 2315 m, 5 Jul 1924, *I. M. Johnston* s.n. (GH, NY n.v.); Same site, 5 Jul 1924, *F. W. Peirson* 4902 (DS, RSA); Same site, 15 Jul 1976, *R. F. Thorne* 47806 & *L. DeBuhr*, *C. Davidson*, *C. W. Tilforth* (BRY, MICH, NY n.v., RSA); Same site, 5 Aug 2010, *P. F. Zika* 25278 & *N. Otting*, *S. Eliason*, *L. J. Gross* (WTU); Same site, 1 Jul 2017; *D. S. Bell* 11142A (CAS, RSA, WTU); Same site, 1 Jul 2017; *D. S. Bell* 11145 (RSA); San Bernardino Mountains, NE of Hitchcock Springs, 2286 m, 7 Jun 2011, *L. J. Gross* 5489 & *L. Ward* (RSA, SD, SFV, WTU); Little Bear Springs campground, near the Pacific Crest Trail, 1994 m, 16 Jun 2011, *L. J. Gross* 5526 & *J. Paolini*, *L. Ward* (RSA); eastern fork of Knickerbocker Canyon, 2314 m, 26 Jul 2011, *L. P. Janeway* 10578 (CHSC n.v., OSC n.v., RSA, WTU); head of Little Morongo Canyon, 2562 m, 15 Jun 2016, *D. S. Bell* 9734 & *L. J. Gross* (MO, RSA, WTU); San Bernardino Moun-

tains, Fish Creek in the upper Santa Ana River watershed, 2166 m, 25 May 2016, *D. S. Bell* 9642 & *J. England* (CHSC, MICH, RSA, WTU); San Bernardino Mountains, above Dry Lake, 2776 m, 31 May 2016, *D. S. Bell* 9670 & *L. J. Gross* (CHSC, RSA, WTU); San Bernardino Mountains, recently burned rocky ridge between North Fork and South Fork of Mission Creek, 2452 m, 14 Jun 2016, *D. S. Bell* 9698 & *L. J. Gross* (OSC, RSA, WTU); San Bernardino Mountains, San Gorgonio Wilderness, near the head of the South Fork of the Whitewater River, 2369 m, forest openings, on semi-shady benches above creek, 18 May 2017, *D. S. Bell* 10810 & *L. J. Gross* (RSA, SD, UCR, WTU); Same site, 22 Jun 2017, *D. S. Bell* 11096 & *A. Chambers* (JEPS, RSA, WTU).

DISTRIBUTION AND ECOLOGY

Carex cryptosperma is endemic to southwestern California. It was first collected in 1924 by I. M. Johnston and F. W. Peirson, both at Bluff Lake on 5 July. The known sites (Fig. 2) are all within the San Bernardino and San Gabriel Mountains of the Transverse Ranges, excepting a single locality in the San Jacinto Mountains in the Peninsular Ranges (Baldwin et al. 2012). In aggregate, the known populations encompass an area 90 km from east to west and 60 km from north to south. Elevations range from 1630–2775 m, on flat or gentle sandy to rocky slopes, often in pine duff. The underlying bedrock, where recorded, is igneous granodiorite. Most collections are from dry open pine forest, rocky ridges, or benches above riparian zones, in partial shade, or in full sun where forest cover was removed by a recent fire. The species appears to be somewhat fire tolerant; the surviving plants can be vigorous in the year following a blaze, presumably due to a flush of nutrients released into the soil.

Like other members of *Carex* sect. *Acrocystis*, the flowers appear to be wind-pollinated, and the swollen arillate perigynium stipe likely serves as an ant attractant to facilitate dispersal (Lengyel et al. 2010).

Associated species include: *Abies concolor*, *Acmispon argyraeus* (Greene) Brouillet, *Amorpha californica* Nutt., *Arctostaphylos patula* Greene, *Artemisia dracunculus* L., *A. ludoviciana* Nutt., *Boechera pinnatorum* (Tidestr.) Windham & Al-Shehbaz, *Calocedrus decurrens* (Torr.) Florin, *Calochortus invenustus* Greene, *Carex abrupta* Mack., *C. multcostata* Mack., *C. rossii*, *Castilleja applegatei* Fernald, *Ceanothus cordulatus* Kellogg, *Cercocarpus betuloides* Nutt., *C. ledifolius* Nutt., *Elymus elymoides* (Raf.) Swezey, *Eriodictyon trichocalyx* A.Heller, *Eriogonum fasciculatum* Benth., *E. parishii* S.Watson, *E. wrightii*, *Eriophyllum confertiflorum* (DC.) A.Gray, *Erysimum capitatum* (Douglas ex Hook.) Greene, *Fremontodendron californicum* (Torr.) Coville, *Gayophytum diffusum* Torr. & A.Gray, *Geranium richardsonii* Fisch. & Trautv., *Hieracium albiflorum* Hook., *Leptosiphon breviculus* (A.Gray) J.M.Porter & L.A.Johnson, *L. melingii*, *Linanthus pungens* (Torr.) J.M.Porter &

L.A.Johnson, *Lupinus excubitus* M.E.Jones var. *austromontanus* (A.Heller) C.P.Sm., *Monardella linoidea* A.Gray, *Nama rothrockii* A.Gray, *Oenothera californica* (S.Watson) S.Watson, *Pedicularis semi-barbata* A.Gray, *Penstemon grinnellii* Eastw., *P. labrosus* (A.Gray) Hook. f., *P. rostriflorus* Kellogg, *Pinus contorta*, *P. jeffreyi*, *P. lambertiana* Douglas, *Poa fendleriana* (Steud.) Vasey, *P. pratensis* L., *P. secunda* J.Presl, *Populus tremuloides*, *Pteridium aquilinum* (L.) Kuhn, *Quercus chrysolepis*, *Q. kelloggii* Newb., *Ribes nevadaense* Kellogg, *Rosa californica* Cham. & Schltdl., *Salvia pachyphylla* Epling ex Munz, *Solanum xanti* A.Gray, *Solidago californica* Nutt., *Sphenosciadium capitellatum* A.Gray, *Stipa parishii* Vasey, *Symphoricarpos rotundifolius* A.Gray, *Tetradymia canescens* DC., and *Vulpia microstachys* (Nutt.) Munro.

PHENOLOGY

Carex cryptosperma fruits were collected between 17 May and 7 July. Flowering material was inseparable from other members of *Carex* sect. *Acrocystis*.

ETYMOLOGY

Carex cryptosperma, or cryptic sedge, is named for the hidden seeds, achenes, perigynia, and spikelets.

As is true for many members of *Carex* sect. *Acrocystis*, the basal spikelets, obscured by the foliage, are easy to overlook in the field.

SIMILAR SPECIES

Carex cryptosperma is assigned to *Carex* sect. *Acrocystis* (Crins and Rettig 2002). It is similar to *Carex rossii*. We note that the two grow near each other at Bluff Lake, but we have not found mixed collections in the herbarium. Both form thick low clumps, have a long leaf-like inflorescence bract, and have a perigynium stipe shorter than (or equal to) the beak. They are best distinguished by the lateral nerves on the pistillate scales and the nerved faces of the perigynia for *C. cryptosperma*. In *C. rossii*, the perigynia have two coarse marginal ribs, but no heavy nerves on the faces, and the pistillate scales generally lack faint lateral nerves flanking the midvein.

The ratio of the perigynium beak length to stipe length differs between *Carex cryptosperma* and *C. bajacalifornica*. *Carex cryptosperma* has a stipe shorter than (or equal to) the prominent beak. *Carex bajacalifornica* has a stipe longer than (sometimes twice as long as) the short beak.

KEY TO *CAREX* SECT. *ACROCYSTIS* OF NORTHWESTERN MEXICO AND CALIFORNIA, BASED ON ZIKA ET AL. (2012)
We suggest collecting and keying material with ample and fully mature perigynia.

- 1. Fertile stems entirely pistillate or entirely staminate; habitat serpentine deposits of southwestern Oregon and Del Norte Co., California *C. serpenticola*
- 1' Fertile stems (or at least the elongate ones) bearing both pistillate and staminate flowers
- 2. Basal spikes absent, all spikes on elongate stems
- 3. Pistillate scales and proximal staminate scales green, red, or purple with hyaline margins 0.4–0.8 mm wide; terminal spike staminate; stems always monoecious, habitat never serpentine bedrock, southern British Columbia to Trinity Co., California *C. inops* L.H.Bailey subsp. *inops*
- 3' Pistillate scales and proximal staminate scales dark purple with hyaline margins 0.1–0.2 mm wide; terminal spike variable, staminate, pistillate, or gynecandrous; stems monoecious or dioecious; habitat serpentine deposits, southwestern Oregon and Del Norte Co., California *C. serpenticola*
- 2' Basal spikes present, also usually with some spikes on elongate stems
- 4. Perigynia veinless or nearly so except for two strong marginal ribs; pistillate scales with 1(–3) prominent midveins, often lacking fine parallel lateral veins
- 5. Inflorescence bracts inconspicuous and shorter than the inflorescence on elongate stems (occasionally shoots produced in a second flush of growth will have elongate inflorescence bracts); proximal sheaths disintegrating into stiff fibers; perigynia 1.5–2.1 mm wide; coastal dunes and headlands, southern Vancouver Island, British Columbia, to San Luis Obispo Co., California. *C. zikae* Roalson & Waterway
- 5' Inflorescence bracts conspicuous and usually longer than the inflorescence on elongate stems; proximal sheaths not disintegrating into stiff fibers; perigynia 1–1.7 mm wide; widespread, coastal and montane to alpine; the taxa of lead 6 sometimes difficult to separate
- 6. Perigynia 3.1–4.5 mm long, beaks 0.7–1.7 mm long, beak teeth 0.2–0.5 mm long; stems usually ascending; habit loosely to densely cespitose; rhizomes often stout, 1.1–3 mm diameter; sea level to alpine, central Alaska to northern Baja California, Mexico, east to Michigan and New Mexico *C. rossii*
- 6' Perigynia 2.3–3.1 mm long, beaks 0.4–0.8 mm long, beak teeth 0.1–0.2 mm long; stems usually spreading or arching; habit loosely cespitose; rhizomes often slender, 0.8–2.0 mm diameter; montane and alpine, British Columbia to the San Bernardino and San Gabriel Mountains of southern California, east to Alberta and Colorado. *C. deflexa* var. *boottii*
- 4' Perigynia with 3–5 veins on at least one face, usually extending to mid-body or beyond, in addition to the two marginal ribs; pistillate scales with 1–5 prominent midveins, sometimes with up to 14 less conspicuous parallel lateral veins

- 7. Foliage blue-green when fresh, strongly papillose abaxially when dry, at least on new growth (40×); perigynium body elliptic or barrel-shaped; Lane Co., Oregon to San Luis Obispo Co., California, and disjunct in San Diego Co. *C. brainerdii* Mack.
- 7' Foliage green when fresh, smooth to scabrous abaxially when dry, never papillose; perigynium body obovoid to subglobose
- 8. Some basal pistillate spikes on long weak ultimately arching peduncles more than 10 cm long; perigynia up to 2.3 mm wide; perigynium stipes up to 2.2 mm long when dry; coastal mesic forest and meadows, California endemic, Del Norte Co. to Channel Islands, and to 1500 m elev. in San Gabriel Mountains and San Diego Co. *C. globosa*
- 8' All basal pistillate spikes on stiffly erect peduncles less than 8 cm long; perigynia up to 1.9 mm wide; perigynium stipes up to 1.6 mm long when dry; habitat inland to montane, dry forest, savanna and chaparral
- 9. Perigynium stipes shorter than or equaling beaks; perigynium beaks 1.0–1.3 mm long; fruiting in May and June; 1630–2775 m elev., San Gabriel, San Jacinto and San Bernardino Mountains, southern California *C. cryptosperma*
- 9' Perigynium stipes longer than beaks; perigynium beaks 0.5–0.9 mm long; fruiting from late March to early June; 450–2670 m elev.; not known from southern California
- 10. Stems 9.5–35 cm tall; basal spikes on peduncles 10–75 mm; perigynia 3.4–4.9 × 1.4–2.1 mm; gabbro and serpentine meadows, chaparral, and open forest, 450–770 m elev.; northern Sierra Nevada foothills, Butte to El Dorado Cos., California *C. xerophila*
- 10' Stems 1–6.5 cm tall; basal spikes sessile or on peduncles 1–5 mm; perigynia 2.9–3.7 × 1.2–1.6 mm; open pine forests, 1650–2670 m elev.; northern Baja California, Mexico *C. bajacalifornica*

ACKNOWLEDGMENTS

We are grateful to the museum staff at herbaria that assisted us or provided images or loans: BCMEX, BRY, CAS, CHSC, DS, GH, MICH, NCU, NY, POM, RSA, SD, SFV, UCR, and WTU. David Giblin, Jon Rebman, and Andy Sanders assisted in many ways. No study of the *Acrocystis* is possible without lengthy discussion of the morphological subtleties, and for this we are indebted to Dick Brainerd, Bill Crins, Bruce Ford, Lawrence Janeway, Max Licher, Nick Otting, Tony Reznicek, Glenn Rink, and Barbara Wilson. The late Reid Moran’s explorations of Baja were the starting point, and we remember him for his humor and generosity with his time. Three anonymous reviewers provided many useful suggestions and we thank them for their contributions. The plates were prepared by Crystal Shin; the map by Tom M. Ruehli; both were a pleasure to work with. Jan Kirschner kindly provided the Latin diagnoses. For assistance in the field we extend our appreciation to Andrew Chambers, Robin Eliason, Scott Eliason, Joy England, Marta Lefevre-Levy, Joshua Paolini, Lindsay Ward, and the Mountaintop District, San Bernardino National Forest.

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2019 GRADUATE STUDENT SYMPOSIUM AT CAL POLY, SAN LUIS OBIPSO

Save the Date! The 2019 Annual Banquet & Mixer and the semi-annual Symposium of the California Botanical Society will be held at the California Polytechnic State University, San Luis Obispo on Saturday, April 6, 2019. The CBS Council is delighted to report that Dr. David Lowry (Professor in the Department of Plant Biology, Michigan State University) will be delivering the evening's presentation entitled "Mechanisms of local adaptations to California's coastal and inland habitats in the yellow

monkeyflower." Details regarding the symposium's time and location, registration and payment, and recommendations for lodging will be announced on the CBS website (calbotsoc.org). Field trips will also be offered during the weekend of the symposium.

—SUSAN J. MAZER President, California Botanical Society, Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, 93106, mazer@lifesci.ucsb.edu.

CBS President's Report for Volume 65

Dear Colleagues,

Given the delay between the time of this writing and its appearance in *Madroño*, it may surprise you to read that I just returned from Botany 2018 (July 21–25), the annual conference of the Botanical Society of America (BSA), co-sponsored by the American Society of Plant Taxonomists, the American Fern Society, the Society of Herbarium Curators, the Canadian Botanical Association, and the International Association of Plant Taxonomists. More than 1000 plant biologists attended the meeting and, as usual, it was a source of inspiration, information, simultaneously scheduled not-to-be-missed talks, encounters with old friends, introductions to new ones, and not a little bit of nostalgia. Forty of the 301 published abstracts included the word “California” — a great showing that reflects the state's strong history as a botanical hotspot. Authors from almost 70 institutions in western states presented papers or posters.

The BSA conference in 1981 was the first meeting that I ever attended, delivering my first talk (with index cards, diazo blue slides, and shaking knees) the summer after I graduated from college. Recalling how nervous I was during this presentation, it is impossible not to be impressed by how poised, engaging, and charismatic many of the youngest presenters were at this year's meeting. And, it still thrills me to be at a meeting surrounded by fellow botanists, all taking unbridled delight in plants and not needing to explain why. Speaking of whom, the annual California Botanists lunch — an informal gathering of attendees who either have conducted, or are conducting, research in California — was particularly well attended this year, and we were all very pleased to welcome two recently hired Assistant Professors: Andy Gardner (CSU Stanislaus) and Clayton Visger (CSU Sacramento).

The California Botanical Society was also very busy at this year's BSA meeting. Council Member-at-Large Dr. Josh Der (CSU Fullerton) spearheaded the organization of our exhibit booth, where we offered free memberships (for 2018) to curious visitors, many of whom also nominated their students for membership and/or expressed interest in participating in our Botany Ambassador Program, either as reviewers for *Madroño* or as writers of non-technical translations of *Madroño* articles for our newsletter (*Nemophila*) and website (<https://www.calbotsoc.org>). If these activities interest you or your students, please contact our Outreach Coordinator, Danielle Black (danielleblack@lifesci.ucsb.edu), to learn how to participate. Many thanks are due to Second Vice President, Dr. Jenn Yost (California Polytechnic

University, San Luis Obispo, aka Cal Poly SLO) and Dr. Matt Ritter (Cal Poly SLO and former *Madroño* Editor), who spent many hours at our booth helping to promote the Society.

As we watched neighboring exhibition booths sell T-shirts hand-over-fist, we realized that perhaps there's a boat out there that we've been missing. To wit: if you wish to offer your own original design for a California Botanical Society T-shirt, please send a high-resolution graphical image to Danielle (danielleblack@lifesci.ucsb.edu). I promise you that the Council will consider it in time to order T-shirts and to sell them at our 2019 Graduate Student Symposium. Possible uses of the proceeds include increasing the number and award size of our annual Paul Silva Student Research Grants or for the Annetta Carter Memorial Fund, and offering more travel subsidies for graduate students attending our semi-annual Graduate Student Symposium.

If you've read my recent messages (letters and the emails) to Society members, you may recall that throughout 2018 we've been offering free first-year memberships to graduate students, postdocs, and recently hired (untenured) faculty and herbarium staff interested in Western U.S. botany. This membership drive has been so effective that we've welcomed more than 125 new members (not including 48 that signed up at Botany 2018) and, perhaps not coincidentally, we've seen a large increase in the number of applicants for the Paul Silva Student Research grants. While we can no longer claim that this grant competition has a 27% success rate (in 2018, we could only fund 3 of 17 applications, or 17%), the quality of the applications was uniformly high, and (if, for example, we sell enough T-shirts), we can hope to increase the success rate in 2019. To learn what our awardees are up to, please read the abstracts of their projects (<https://calbotsoc.org/grants/>) and learn about Nicole Kollars' plans to examine the effects of grazing on the genetic diversity of seagrass (UC Davis); Audrey Hanes' study of night-time transpiration in parasitic plants (UC Berkeley), and Emily Cox's investigation of the effects of water stress in California goldfields (UC Berkeley).

Now is the time to start planning your and your students' participation in the 2018 Graduate Student Symposium, which will be held at Cal Poly SLO on Saturday, April 6, 2019. Co-organizers of the event are Dr. Jenn Yost and Dr. Dena Grossenbacher, and given Cal Poly's history as a hotbed for the study of botanical diversity, ecology, and evolution, I know that we can look forward to a compelling suite of field trips before or after the meeting and banquet

(Come early! Stay late!). Due to popular demand following last year's well-attended banquet at UC Davis, we will aim to provide more time for attendees to socialize and to catch up with each other. Please mark your calendars for this event and encourage your students to participate; it's such a great way for them to build their network of like-minded and lifelong colleagues.

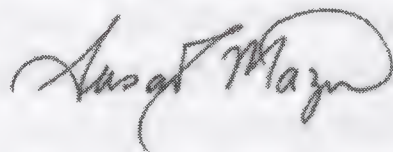
As I reflect on the past year, I'd like to acknowledge a few other Society activities and accomplishments. First, due to the heroic efforts of *Madroño* Editor-in-Chief Dr. Justen Whittall, for the first time we offered eight cash prizes (and award certificates) to students presenting botanical projects at the Science & Engineering Fair in Santa Cruz County and at the 2018 Synopsys Championship in Santa Clara County. Justen anticipates that once the word spreads that plant-centric projects can win cash, we'll see a Green Wave of botanical interest among middle and high school students. If you are interested in participating as a cash-awarding judge in your county's Science Fairs, please contact Danielle Black (danielleblack@lifesci.ucsb.edu), and she'll provide you with judging forms, a template for the award certificates, and instructions for delivering cash awards to students.

Second, as part of our ongoing Botany Ambassador Program, designed to provide public outreach and professional development opportunities for graduate students and postdocs, we have posted on our website 17 K-12 lesson plans that are now available for use in schools, museums, and botanic gardens (<https://calbotsoc.org/outreach/>). If any of you or your students have written botanical lesson

plans for K-12 that you would like to share with us, or know of other publicly available instructional resources that are well-suited to botanical education in California please contact Rachael L. Olliff Yang (membership@calbotsoc.org).

Third, the Council would like to remind all of you that *Madroño* remains a superb, indexed, and widely accessible journal for publishing your research on any botanical topic focused on Western North America. The Editorial Board makes a strong effort to provide rapid turn-around times during the review process and straightforward instructions for manuscript preparation (<https://calbotsoc.org/author-instructions/>). In addition, the journal has a particularly wide readership, not only among botanists in academia, but also among state and federal agency botanists and consultants involved in many wildlife management decisions. Perhaps most importantly, at a time of escalating publication costs, we've also continued to provide 5 free publication pages for members each year. Please encourage your students to consider *Madroño* as a venue for their research, and if you haven't published in *Madroño* yourself, we're ready to receive your manuscripts!

Until our paths cross again, have a great winter and, when in doubt, botanize!



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EDITOR'S REPORT FOR VOLUME 65

I am pleased to report the publication of *Madroño* volume 65 by the California Botanical Society (CBS) for 2018.

In my first year editing *Madroño* solo, I have attempted to keep *Madroño* on track and at the leading edge of scientific, peer reviewed publications in natural history. Highlights from this past year include publishing fifteen full-length Articles, nine Noteworthy Collections and the descriptions of five New Species. The first four Open Access articles in *Madroño* history appeared in volume 65 and quickly became the top *Madroño* articles downloaded in the last 12 months. They are works by Smith and Kay (*Monardella*), Burge et al. (*Arctostaphylos*), Potter et al. (*Juglans*), and Olliff-Yang et al. (*Boechera*).

The average time from submission to publication for *Madroño* remains steady at 8 months. We believe that *Madroño* is still the best outlet for western botanists to publish their discoveries in a timely fashion, while reaching an interested and relevant audience. We hope to grow *Madroño* in the coming year with incentives like faster times to online publication, additional Open Access opportunities, reduced printing charges for color plates and figures, and fostering the next generation of *Madroño* authors by including the Botany Ambassadors as

supplemental reviewers whenever appropriate (see President's Letter in this issue for complete description of the program).

The efforts of numerous individuals are critical to the continued quality of the journal. Thank you to the Noteworthy Collections editor, David Keil. Steve Timbrook has long provided the Volume Index and Table of Contents for the journal and we thank him for his efforts. We thank Lynn Yamashita for providing administrative support throughout the year. Lynn, with the help of Rachael Olliff-Yang, ensure that your *Madroño* hardcopies arrive at the correct address with informative inserts. We are also grateful to our reviewers who regularly provide insightful comments bettering our publication. They are individually acknowledged in this issue's "Reviewers of Manuscripts" section.

Editing *Madroño* is a joy. Being on the forefront of western botany and working with so many wonderful botanists as authors and reviewers is a pleasure. Botany is alive and well in western North America! The evidence is in *Madroño*. Please continue to submit your work to the journal, recruit new authors, and have a great year.

Justen Whittall

November 2018

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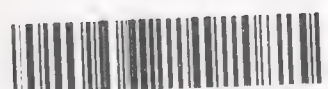
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